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1 Biochar as a growing media component for containerized production of Douglas-fir

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Draft

9 ABSTRACT

10 In the Inland Northwest US, Douglas-fir artificial regeneration commonly includes growing
11 seedlings in media containing sphagnum peat. Concerns over the sustainability of peat and
12 rising plant production costs are initiating investigation of growing media alternatives. Biochar
13 is a potential media amendment that has positive physical and chemical properties for seedling
14 production, including high water and nutrient retention due to large surface area, which may
15 reduce leaching losses and improve fertilizer use efficiency. We used different amounts of
16 biochar to amend peat-based growing media to determine if seedling growth response to various
17 fertilizer rates differed with biochar amendments. Every 13 weeks for 39 weeks replicate
18 seedlings were measured for photosynthetic activity, destructively harvested, and analyzed for
19 leaf N concentration. Biochar did not reduce fertilizer rates required to grow equal sized
20 seedlings nor improve seedling growth. When mixed with peat at rates of 25% or 50% by
21 volume, biochar progressively reduced height and diameter growth rates, seedling biomass, and
22 photosynthetic rate. Biochar increased growing media pH to levels incompatible with conifer
23 seedling requirements, decreased media extractable P concentration, which may have caused
24 decreased photosynthesis. Adjusting pH of the biochar used would be necessary to grow
25 Douglas-fir seedlings for forest regeneration.

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30 KEY WORDS

31 conifer, fertilizer, peat, pH, photosynthesis

32 INTRODUCTION

33 In 2015, over 130,000 conifer hectares were planted in Idaho, Oregon, and Washington,
34 and over 61 million seedlings were produced from containerized growing stock (Hernandez et al.
35 2016). Interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco)
36 is an important and highly valuable timber species in the region. Artificial regeneration of
37 Douglas-fir is used in place of natural regeneration to improve genetics and assure stocking
38 (Hermann and Lavender 1990).

39 Containerized growing stock utilizes irrigation, fertilizer application, and artificial
40 growing media in a nursery setting. Sphagnum peat is often a major growing media component
41 because it has many desirable features including high water-holding capacity, high cation
42 exchange capacity (CEC), and high air capacity at maximum water content (Landis et al. 1990;
43 Nelson 2012). It has low bulk density, pH, microbe activity, and nutrient content. Sphagnum
44 peat is free of weed seeds and pathogens, and it is homogenous.

45 Recently there has been environmental and economic concerns with the use of peat. Peat
46 bogs are important habitats and carbon (C) stocks, which provide environmental services such as
47 local water quality regulation and flood protection (Alexander et al. 2008). Peat extraction has
48 negative environmental impacts (Alexander et al. 2008) as peat usually acts as a C sink, but
49 when a peatland is drained and extracted for other uses the peat decomposes quickly and emits
50 greenhouse gases (Cleary et al. 2005). Therefore, there is a search for alternatives to using peat
51 as a component of containerized growing media (Abad et al. 2001).

52 One possible alternative or amendment to peat is biochar. Biochar is charcoal created via
53 pyrolysis (Bridgewater 2004), but is defined by its intentional soil application for environmental
54 uses (Lehmann and Joseph 2009). Biochar has some of the same positive physical attributes of

peat. Biochar has low bulk density (Blok et al. 2017), high total air space (Blok et al. 2017), and good water retention (Laird et al. 2010). When mixed with peat, biochar increases air space, water-holding capacity, and total porosity (Mendez et al. 2015) and when pelleted, biochar added to peat improves hydraulic water conductivity and water availability (Dumroese et al. 2011). Biochar-amended peat substrate has higher nutrient concentrations (Nemati et al. 2015), increased pH (Nair and Carpenter 2016) and increased CEC (Headlee et al. 2014) compared to peat substrates without biochar amendment. Biochar added to a peat and perlite media provides available nutrients to the growing media (Locke et al. 2013) and when wood ash is added, the wood ash addition increases foliar P and K concentrations of plants grown in the peat media (Hytonen 2016). Biochar's ability to retain nutrients could mean less fertilizer needed to grow plants especially because charcoal has retention properties that prevent fertilizer ingredients from leaching (Glaser et al. 2002).

Many studies include biochar in conjunction with fertilizer. Biochar without fertilizer does not provide enough P or K for container crops (Locke et al. 2013). Biochar with fertilizer has both positive and negative effects on plant growth. When added to field soils, biochar both increases crop fertilizer N uptake (Huang et al. 2014), and growth (Albuquerque et al. 2013). Biochar also causes plant biomass production to react both positively and negatively when combined with fertilizer application, dependent on soil type (Van Zwieten et al. 2010). We are not aware of published research pertaining to biochar crossed with different fertilizer rates to assess plant performance in a peat substrate, especially in tree seedlings.

Pre-treatment of biochar with nutrients before use promotes plant growth by increasing the percentage of functional chemical groups that create electrostatic charge for binding cations and improves nutrient availability to plants (Joseph et al. 2013). Fertilizer- or urine-treated

biochar increases crop yield compared to those receiving only chemical fertilizer (Joseph et al. 2013) or only urine (Schmidt et al. 2015). Crop yield increases are attributed to biochar's improved capacity to capture and exchange plant nutrients. However, pre-treatment doesn't always mean increased growth (e.g. Nielsen et al. 2014). We are unaware of studies on the use of nutrient pre-treated biochar in peat-based growing media or its use to grow tree seedlings.

Biochar is often used in agriculture, but some work has been done in forest settings (Page-Dumroese et al. 2016). Mixed wood ash (mixture of fly ash and charcoal) amended to *Pinus radiata* plantations increases tree growth (Omil et al. 2013). Boreal angiosperm tree seedlings grow better than gymnosperm seedlings with biochar amendment (Pluchon et al. 2014). A meta-analysis that largely included seedling data found that there is a potential for an average of a 41% increase in tree growth response to biochar (Thomas and Gale 2015).

Biochar can be used with growing media to grow plants. Most work in this realm relates to horticultural or agricultural crops. Biochar added to growing media improves growth of tree scions, fruits, and vegetables due to improved hydrophysical properties and improved nutrition (De Tender et al. 2016; Headlee et al. 2014; Mendez et al. 2015). Hybrid poplar (*Populus spp* L.) growth increases with biochar amendment may be due to higher K availability (Headlee et al. 2014). Improved nutrition or water properties does not always explain increased growth response for vegetables, but growth increases from biochar could be due to stimulation of beneficial plant growth microbes or hormesis (Graber et al. 2010; Nieto et al. 2016). Locke et al. (2013) saw no signs of nutrient deficiency with biochar addition and no effect of biochar on plant growth. Biochar may also decrease growth due to increasing substrate pH (Nair and Carpenter 2016). High pH is known to affect nutrient availability (Lucas and Davis 1961), which may, in turn, affect plant photosynthesis (Pallardy 2008), resulting in less plant growth. We are unaware

of reports on temperate conifer seedlings grown in growing media amended with biochar to test these potential impacts on plant growth and physiology.

In this study, we used biochar from a mixed conifer feedstock as an amendment to a peat-based media to grow Douglas-fir seedlings intended for forest regeneration. We used different amounts of biochar that was either pre-treated with fertilizer or not and we applied different rates of fertilizer in a forest nursery setting. Our objectives were to determine if biochar is a suitable amendment to peat-based media and if seedling growth response to fertilizer differs with biochar amendments. Based on previous research showing biochar's ability to retain nutrients, we hypothesize that biochar amendment will increase growth at a given fertilizer level, especially with pre-treated biochar. To account for seedling growth responses, we measured seedling biomass and seedling photosynthetic rate.

MATERIALS AND METHODS

Media

The media consisted of biochar, either pre-treated with liquid fertilizer or left untreated, a peat-based potting mix, and time-release fertilizer in different proportions. Evergreen Forest Products biochar (New Meadows, ID) was used in this experiment. Evergreen Forest Products biochar is produced from Western mixed conifer feedstock (largely ponderosa pine mill residue) in a steam boiler at 980 °C. It was chosen because hybrid poplar grew best in peat-based media that was amended with Evergreen Forest Products biochar when compared with three other types of biochar (Coleman and McDonald, unpublished data). It was unique among the others in that it had relatively low C (25.7%), high ash (40.3%), and high surface area (201 m² g⁻¹). Biochar was applied at three rates (0, 25, and 50% by volume). These rates were chosen to represent an operationally relevant replacement for peat known to be favorable for plant growth. Headlee et

al. (2014) found 25% biochar to be a suitable rate and Mendez et al. (2015) found 50% to be suitable for growing plants in peat-based growing media. Half of the biochar was pre-treated with Peters Professional Soluble Plant Food 20-20-20 (The Scotts Company LLC, Marysville, OH) for use in the Treated biochar treatment. Treated biochar was soaked in 100 mg N L⁻¹ fertilizer and then rinsed 3 times. The other half of the biochar was left untreated with fertilizer pre-treatment and was also rinsed 3 times. After treatment and rinsing, pre-treated biochar contained 2.9 mg N g⁻¹ compared to 1.8 mg N g⁻¹ for untreated biochar. A standard nursery peat potting mix (Canadian Sphagnum peat, vermiculite, and fine aged bark) (SunGro, Agawam, MA) was used to replicate standard nursery practices at the University of Idaho Pitkin Forest Nursery (Moscow, ID, 46.7254° N, 116.9560° W). The surface area of this peat-based potting mix was small (1.16 m² g⁻¹) compared to the biochar used. Polymeric-resin coated fertilizer (Osmocote, 15-9-12 NPK, plus micronutrients, Scotts Company, Marysville, OH) was applied based on product recommendations at full rate, (0.790 g N L⁻¹), half rate, (0.395 g N L⁻¹) or quarter rate (0.198 g N L⁻¹). Treatment components (biochar, potting mix, and fertilizer) were blended in a 170 L cement mixer for 10 minutes. Once mixed, the treatments were manually loaded into 45-cell Styroblock containers (Tangent, OR). Each individual cell had a volume of 340 ml. Each treatment consisted of two, 45-cell Styroblock containers, for a total of 90 seedlings per treatment from which representative individual seedlings, taken equally from both containers, were used for various measurements. The study consisted of 18 unique treatments, see Figures S1¹ and S2² for Styroblock container layout and treatment combinations. Mechanically seeded

¹ Supplementary Fig. S1 is available with the article through the journal website at

² Supplementary Fig. S2 is available with the article through the journal website at

containers were manually top-dressed with forest nursery grit (Target[®] Forestry Nursery Grit, Burnaby, BC, Canada) following standard Pitkin Nursery practices.

Seedlings and Nursery Conditions

In March 2016 interior Douglas-fir seedlings were sown in triplicate in each cell. If more than one seed germinated, all were removed but the most vigorous seedling. Seedlings were grown under standard Pitkin Nursery light and temperature conditions. During the germination and establishment stage (weeks 0-13), average maximum temperature was 27.3 °C and average low was 17.5 °C. During the period of active growth (weeks 13-26), average maximum temperature was 28.9 °C and average low was 16.4 °C. In the hardened off stage (weeks 26-39), average maximum temperature was 18.6 °C and average minimum temperature was 6.4 °C. Photoperiod varied with day length. Containers were placed on one bench in the greenhouse and their positions were randomly rearranged monthly. Seedlings were watered based on a weight loss method (Dumroese et al. 2015). Once designated weigh containers (chosen from containers with the 25% biochar rate) contained 85% of their initial weights, they were watered via overhead irrigation. Irrigation water was injected with phosphoric acid (0.175 ml L⁻¹) to lower the pH and R11 (Wilbur-Ellis, Aurora, CO) was also added as a surfactant. Once budset occurred (September 2016), watering was reduced to 70-75% of initial weight.

Measurements

Ten representative seedlings were measured biweekly for height and diameter growth. Height measurement began at week 6 and diameter measurements began at week 14. Diameter measurements were delayed to insure measurements would not harm tender young seedlings.

Seedlings were harvested three times throughout the experiment with different representative seedlings. Harvests occurred at weeks 13 (germination and establishment), 26

167 (active growth), and 39 (hardened off). Ten seedlings from each treatment were taken at each
168 harvest. After measuring height and diameter, seedlings were dissected into root, stem, and
169 needle components, oven dried at 60 °C, and weighed.

170 *Gas Exchange*

171 Before each harvest, seedlings were measured for photosynthetic gas exchange using a
172 LICOR 6400XT portable Photosynthesis System (LICOR, Lincoln, NE) with a lighted conifer
173 chamber. Conditions in the chamber were controlled at 20 °C temperature, 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$
174 photosynthetically active radiation, 410 $\mu\text{mol CO}_2$, and 45-50% humidity. When using a conifer
175 chamber, the contained leaf area varies among seedlings and therefore, that leaf area needs to be
176 measured independently for each seedling. Needles in the chamber were detached and
177 photographed after gas exchange measurements. Photographs were used to determine leaf area
178 of needles using ImageJ software (Abramoff et al. 2004). Needle dry mass was collected after
179 oven drying to constant weight at 60 °C. Needles were then ground to a fine powder using a
180 WIG-L-BUG mixer (3110-3A, Dentsply-Rinn, York, PA) and were analyzed for C and N
181 concentration using a Costech elemental analyzer (ESC 4010, Costech Analytical, Valencia,
182 CA). At the first harvest the seedlings were very small, so different seedlings were selected from
183 the treated Styroblock and used for either gas exchange or for destructive harvest. All seedlings
184 within a treated Styroblock are representatives of that treatment. For the second and third
185 harvests, the same seedlings that were measured for gas exchange were also destructively
186 sampled for biomass.

187 *Growing Media Chemical Analysis*

188 Growing media from four seedling cells of each treatment was collected after 39 weeks.
189 Roots and slow-release fertilizer prills were removed from the growing media before analysis.

The growing media was analyzed for pH, electrical conductivity (EC), and nutrient concentrations using a Saturated Media Extract (SME) method (AgSource Laboratories, Lincoln, NE) following methods for testing growing media (Warncke 1988). Briefly, ~400 ml samples of growing media were analyzed without pre-processing. Water was added to the sample until the sample just became saturated and pH was determined directly from the saturated sample. Extract was filtered and P, Ca, and Fe was determined with an inductively coupled plasma emission spectrograph.

Data Analysis

Nitrogen and C content were calculated by multiplying nutrient concentration by dry mass. Nitrogen uptake was calculated as the change of N content from one harvest to the next.

The effect of biochar treatment, biochar rate, fertilizer rate, harvest, and their interactions on growth measurements, photosynthetic rate, and leaf N contents were tested in a four-way factorial analysis using the generalized linear mixed model (PROC GLIMMIX) with SAS Software version 9.4 (SAS Institute Inc, Cary, NC). Post-harvest growing media chemistry was tested with a three-way factorial analysis of biochar treatment, biochar rate, fertilizer rate, and their interactions. The random effect of container number was used in all models. Description of statistical linear models can be found in Tables S1³ and S2⁴. Type III tests of fixed effects were used to examine main effects and their interactions. Differences were considered significant at $p \leq 0.05$. If a significant effect was found, Tukey-Kramer tests were performed for multiple comparisons. When necessary to meet normality and homoscedasticity assumptions for analysis of variance, the data was transformed using either a log or square root transformation.

³ Supplementary Table S1 is available with the article through the journal website at

⁴ Supplementary Table S2 is available with the article through the journal website at

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Correlations of leaf N content with photosynthetic rate and media pH and media extractable P were analyzed using PROC CORR with SAS Software version 9.4 (SAS Institute Inc, Cary, NC).

RESULTS

Growth

Seedling height and diameter growth response to biochar rate depended on fertilizer rate (BxF, Table 1). The 0% biochar full fertilizer rate seedlings grew significantly taller (Fig. 1a) and had larger diameters (Fig. 1b) than all other treatments. Height and diameter growth decreased progressively with increasing biochar rate, especially with full fertilizer. The trend of height and diameter growth rate decreasing with fertilizer rate was evident in the 0% and 25% biochar rates, but not in the 50% biochar rate. At the 50% biochar rate, none of the seedlings differed in height growth rate among fertilizer treatments, but diameter growth did differ between lower fertilizer rates.

Total seedling biomass (g) was influenced by biochar treatment, fertilizer rate, and harvest (TxFxH, Table 1) (Fig. 2). Regardless of biochar treatment, the highest fertilizer rate seedlings were significantly larger than the lowest fertilizer rate seedlings at each harvest. Seedling total biomass did not differ significantly between the full and half fertilizer rates in the treated biochar but did in the untreated biochar during harvest 2. In harvest 2, seedlings grown at the lowest fertilizer rate were larger in treated than those grown with untreated biochar and this is the only occurrence of treated vs. untreated differences at corresponding fertilizer rates.

Photosynthetic (PS) rate was affected by biochar and fertilizer rates, but those responses depended on harvest. Photosynthetic rate decreased from 0% biochar rate to 25% and 50% biochar rate in harvests 1 and 2, but not in harvest 3 (BxH, Table 1) (Fig. 3a). The highest PS

rate occurred in the first harvest for seedlings grown with 0% biochar. There was no effect on PS rate from fertilizer rate in harvests 1 or 3, but in harvest 2, PS rates of the fully fertilized seedlings were significantly larger than those in the lowest fertilizer rates (F_xH, Table 1) (Fig. 3b). Photosynthetic rate was lowest in the third harvest.

Needle N concentrations depended on biochar treatment, biochar rate, fertilizer rate, and harvest. Needles in the full and half fertilizer rates usually had higher N concentrations than those from the lowest fertilizer rate, the main exception to this was in harvest 3 (B_xF_xH, Table 1) (Fig. 4b). Biochar treatment also played a role in needle N concentration, dependent on biochar rate and fertilizer rate (T_xB_xF, Table 1) (Fig. 4a). Needle N concentration in treated biochar was typically higher than needle N concentration in untreated biochar. In general, seedlings in the lowest fertilizer rates had lower needle N concentration than fully fertilized seedlings.

The 25% biochar rate seedlings had the highest foliar N uptake rates, based on increase in N content. Nitrogen foliar uptake during active growth was 20-42% greater in the 25% biochar seedlings compared to 0% and 50% biochar seedlings (Supplementary Fig S3⁵).

Media Analysis

Both media pH and extractable P responded to biochar and fertilizer treatments. There was a significant negative correlation between pH and extractable P concentration ($r^2=0.71$, $p<0.01$) (Supplementary Fig S4⁶). Media pH increased with biochar amendment and depended on fertilizer rate (B_xF, Table 2) (Fig. 5a). The fertilizer effect was strongest with 0% biochar and there was no fertilizer effect on pH at the 50% biochar rate. The extractable P concentration

⁵ Supplementary Fig. S3 is available with the article through the journal website at

⁶ Supplementary Fig. S4 is available with the article through the journal website at

255 changed across biochar and fertilizer rates (BxF, Table 2) (Fig. 6b). Extractable P response to
256 fertilizer varied across biochar rates. With 0% biochar, extractable P decreased with fertilizer
257 rates, but with 25% and 50% biochar amendment, extractable P did not decrease with fertilizer
258 rate. Media with 0% biochar had more extractable P than media with 25% or 50% biochar.
259 Concentration of Ca was dependent on biochar and fertilizer rates. (BxF, Table 2)
260 (Supplementary Fig. S5⁷). Proportional decreases in Ca concentration with decreased fertilizer
261 rate was much larger in 0% biochar than 50% biochar.

262 DISCUSSION

263 *Growth*

264 The results of this study suggest that the biochar used is not an adequate substitute for
265 peat in growing media and does not reduce the amount of fertilizer needed to grow Douglas-fir
266 seedlings for forest regeneration. Biochar rate negatively affected total seedling biomass in this
267 peat-based growing media study. Other researchers find that biochar blended with growing
268 media either increases height of ornamental shrubs (Graber et al. 2010; Tripepi 2013) or
269 decreases height of vegetables (Nair and Carpenter 2016). However, Nair and Carpenter (2016)
270 did not see negative height growth effects from biochar in vegetable transplants until the biochar
271 rate was 40% w/w. Biochar is also known to decrease (Bi et al. 2009; Evans et al. 2011; Gravel
272 et al. 2013), cause no response to (Locke et al. 2013) or increase plant growth (De Tender et al.
273 2016; Graber et al. 2010; Headlee et al. 2014; Nieto et al. 2016; Tian et al. 2012). No
274 mechanisms are offered for decreased growth caused by biochar in the previously mentioned
275 studies.

⁷ Supplementary Fig. S5 is available with the article through the journal website at

The negative growth seen in this study may have been due to increased pH resulting in reduced nutrient availability, increased phytotoxic compounds, or excess water retention caused by the biochar. Conifer tree species grow best in pH values between 5.2 and 6.2 (Binkley and Fisher 2013). That optimal pH range was exceeded with the biochar used in our study (Fig. 5a and S4⁸). Biochar is known to increase pH of peat-based growing media (Evans et al. 2011; Vaughn et al. 2013).

Biochar can increase plant productivity by improving pH (Rondon et al. 2007). Our preliminary study showed poplar responded best to the Evergreen Forest Products biochar used in this study compared with three other types of biochar (Coleman and McDonald, unpublished data). Tripepi (2013) found that the Evergreen Forest Products biochar increases growth in the deciduous shrub syringa/mockorange (*Philadelphus lewisii* Pursh), but it decreases growth in Magilla perilla (*Perilla frutescens* (L) Britton). The Evergreen Forest Products biochar used in our study might have increased the pH too much for Douglas-fir seedlings to grow effectively. Species preferences for low or high pH have long been recognized (Lee 1998). When biochar is added to peat mixtures at rates of 60% and 80% by weight the pH rises well above 7.0, which can negatively affect growth of some plants (Nair and Carpenter 2016). Our pH values even exceeded 7.0 (Fig. 5a). If biochar is used at high rates to grow acid-loving plants in growing media, then the biochar pH will need to be lowered (Novak et al. 2009). Conifer trees specifically, can grow in pH above 7.0 if they get enough Fe, Mn, and P (Binkley and Fisher 2013), which is usually accomplished by using chelates or foliar sprays. Phosphorus availability was low in our biochar-amended growing media (Fig. 5b and S4⁹), which is likely caused by

⁸ Supplementary Fig. S4 is available with the article through the journal website at

⁹ Supplementary Fig. S4 is available with the article through the journal website at

precipitation with Ca at pH above 7.0 (Lucas and Davis 1961). Even though biochar can affect nutrient abundance, either through improving nutrient retention or from the biochar releasing nutrients (Lehmann et al. 2011), the high pH caused by biochar amendment could make nutrients unavailable for plant uptake (Binkley and Fisher 2013).

Phytotoxic compounds and oxygen diffusivity could also reduce growth in biochar-amended media. Higher concentrations of phytotoxic compounds in biochar such as polyaromatic hydrocarbons are detrimental to corn (*Zea mays* L.) growth (Rogovska et al. 2012) and was suspected to cause a reduction in pepper transplant growth (Nair and Carpenter 2016). The Evergreen Forest Products biochar does not contain excessive volatile matter due to high production temperature. Rinsing would also have further reduced phytotoxic effects (Rogovska et al. 2012). Therefore, we conclude that phytotoxic compounds are not likely the cause of negative Douglas-fir growth. Biochar, when pelletized, reduces oxygen diffusivity (Dumroese et al. 2011), which could limit oxygen availability to plant roots. We cannot be certain that the Evergreen Forest Products biochar reduced oxygen diffusivity because oxygen diffusivity was not measured and because we did not pelletize the biochar. Based on the media analysis, it is concluded that the increased pH resulting from biochar amendment is a likely cause for the reduced growth in Douglas-fir seedlings.

Douglas-fir growth results from this experiment could differ from other research because of the type and amount of biochar used in the study. There is broad variation among different types of biochar. Biochar differences can affect how plants respond to biochar amendment (Chan et al. 2008). Biochar properties are dependent on the feedstock and the pyrolysis conditions used to make the biochar (Masek et al. 2013). Biochar made from wood can replace at least 20% of peat without affecting plant growth in potting soil, while biochar made from

nutrient rich feedstocks is not adequate for horticulture use (Blok et al. 2017). In this study we found that Evergreen Forest Products biochar at 25% or 50% v/v does not improve Douglas-fir growth and this is likely due to increased pH. Some plants might prefer a different biochar than other plants, due to their differing pH preferences.

Biochar treatment influenced total seedling biomass in the active growth phase (harvest 2), but not in the other two phases (Fig. 2). During the period of active growth, seedlings in the treated biochar displayed a different response pattern to fertilizer rate than the continually decreasing pattern observed with untreated biochar. The lack of the decreasing pattern in the treated biochar could be due to the pre-treated biochar providing essential anions and cations to the seedlings during the phase of active growth (Joseph et al. 2013). During active growth, seedlings are acquiring more nutrients from the treated biochar than the untreated biochar (Supplementary Fig. S3¹⁰). The nutrient effect may not have been seen during emergence because the seedlings were still small and unresponsive, or during post-dormancy because the seedlings were no longer actively growing.

Gas Exchange

Biochar rate negatively affected PS rate in harvest 1 and in harvest 2, with the higher rate of biochar significantly reducing PS rates (Fig. 3a). Decreased PS rates with increased biochar could result from lower nutrients such as Fe or P in the biochar amended seedlings, both of which can reduce PS rates if deficient (Keller and Koch 1964; Niinemets et al. 2001). Although we don't know for certain if the seedlings grown in biochar-amended peat were Fe deficient, we do know that media-amended with biochar had less extractable Fe compared to media without

¹⁰ Supplementary Fig. S3 is available with the article through the journal website at

341 biochar (Supplementary Fig. S6¹¹), which is expected because Fe availability declines with
342 increasing pH (Binkley and Fisher 2013). Similarly, biochar amended media had lower P (Fig.
343 5b and S4¹²). Phosphorous deficiency decreases photosynthetic capacity in conifer seedlings
344 (Ben Brahim et al. 1996).

345 Fertilizer rate did affect PS rate in harvest 2, during the period of active growth (Fig. 3b).
346 During active growth, the higher fertilizer rate was likely providing more nutrients to the
347 seedlings. When essential nutrients (macro and micro) are limited or imbalanced, PS rates can
348 be lower (Pallardy 2008). Increased PS rate is correlated to leaf N (Xu et al. 2015) and in
349 harvest 2, there was a slight, but significant correlation between PS rate and leaf N content
350 ($r^2=0.12$, $p<0.01$).

351 By harvest 3 there were no differences in PS rates among biochar or fertilizer treatments
352 and the rates were much lower than the other harvests (Figs. 3a, b). Reduced PS rates and lack
353 of differences among biochar or fertilizer treatments could be due to lower temperatures and
354 reduced photoperiod. Photosynthetic rates of gymnosperms decline gradually in autumn
355 (Pallardy 2008) and PS rates often decrease when winter dormancy or frost hardening occurs
356 (Oquist 1983). Another potential reason for reduced PS rates in the third harvest could be
357 associated with leaf age. After leaves are fully expanded, there is a gradual decline in PS which
358 is correlated to reduced stomatal conductance, phosphorylation, and Rubisco quantity (Pallardy
359 2008). The reduced PS rates in harvest 3 could be a combination of both seasonality and age.

360 CONCLUSIONS

¹¹ Supplementary Fig. S6 is available with the article through the journal website at

¹² Supplementary Fig. S4 is available with the article through the journal website at

In conclusion, the experiment did not support our hypotheses that biochar would improve seedling growth and reduce fertilizer requirement. The biochar used in this study did not improve the growth of Douglas-fir seedlings when mixed with a standard peat potting mix at rates of 25% or 50% by volume and it did not decrease the amount of fertilizer required to grow equal sized seedlings. Best growth was seen in treatments with 0% biochar. Poor growth is attributed to growing media pH. Growing media amended with biochar had significantly higher pH values than those with 0% biochar likely causing a decrease in P availability, which may have caused decreased PS rates. Adjusting the pH of biochar will be necessary to use it for growing Douglas-fir seedlings for forest regeneration.

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Table 1. P-values of the measured effects of biochar treatment, biochar rate, fertilizer rate, and harvest for height growth, diameter growth, total biomass, PS rate, and N. Boldface indicates significance at $p \leq 0.05$.

Effect	Height growth (cm wk ⁻¹)	Diameter growth (mm wk ⁻¹)	Total biomass (g)	PS rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	N (mg g ⁻¹)
Biochar treatment (T)	0.15	0.41	0.02	0.89	0.01
Biochar rate (B)	<.01	<.01	<.01	<.01	<.01
Fertilizer rate (F)	<.01	<.01	<.01	0.47	<.01
Harvest (H)			<.01	<.01	<.01
TxB	0.20	0.25	0.25	0.44	0.12
TxF	0.53	0.15	0.01	0.80	0.35
TxH			0.45	0.60	0.91
RxF	<.01	<.01	0.01	0.51	0.04
BxH			<.01	<.01	<.01
FxH			<.01	<.01	<.01
TxBxF	0.29	0.56	0.19	0.77	0.01
TxBxH			0.41	0.60	0.14
TxFxH			0.03	0.80	0.23
BxFxH			0.19	0.28	<.01
TxBxFxH			0.16	0.52	0.91

Table 2. P-values of the tested effects of biochar treatment, biochar rate, and fertilizer rate for the post-experiment media analysis. Boldface indicates significance at $p \leq 0.05$.

Effect	pH	P (ppm)	Ca (ppm)
Biochar treatment (T)	0.09	0.11	0.16
Biochar rate (B)	<.01	<.01	<.01
Fertilizer rate (F)	<.01	0.61	<.01
TxB	0.57	0.08	0.30
TxF	0.12	0.15	0.25
BxF	<.01	<.01	0.01
TxBxF	0.99	0.18	0.52

553 **Fig. 1** Height growth rate (cm wk^{-1}) (a) and diameter growth rate (mm wk^{-1}) (b) of Douglas-fir
554 seedlings depended on biochar rate and fertilizer rates. Bars represent standard error, $n=20$.
555 Bars having the same letter above are not significantly different ($\alpha = 0.05$).

556 **Fig. 2** Total dry biomass (g) of Douglas-fir seedlings depended on biochar treatment and
557 fertilizer rate during each harvest. Bars represent standard error, $n=30$. Bars having the same
558 letter above are not significantly different ($\alpha = 0.05$), specific to each harvest.

559 **Fig. 3** Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of Douglas-fir seedlings depended on harvest and
560 biochar rate (a) and harvest and fertilizer rate (b). Bars represent standard error, $n=60$. Bars
561 having the same letter above are not significantly different ($\alpha = 0.05$).

562 **Fig. 4** Nitrogen concentration (mg g^{-1}) of Douglas-fir seedlings depended on biochar treatment,
563 biochar rate, and fertilizer rate (a) and biochar rate, fertilizer rate, and harvest (b). Bars represent
564 standard error, $n=20$. Bars having the same letter above are not significantly different ($\alpha = 0.05$),
565 inclusive to each biochar treatment (a) or each harvest (b).

566 **Fig. 5** pH (a) and P concentration (ppm) (b) of growing media depends on biochar rate and
567 fertilizer rate. Bars represent standard error, $n=20$. Bars having the same letter above are not
568 significantly different ($\alpha = 0.05$).

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570 **Fig. 1**

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573 **Fig. 2**

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Fig. 3

Fig. 4

Fig. 5

Draft

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Effect	Height growth (cm wk ⁻¹)	Diameter growth (mm wk ⁻¹)	Total biomass (g)	PS rate (μmol m ⁻² s ⁻¹)	N (mg g ⁻¹)
Biochar treatment (T)	0.15	0.41	0.02	0.89	0.01
Biochar rate (B)	<.01	<.01	<.01	<.01	<.01
Fertilizer rate (F)	<.01	<.01	<.01	0.47	<.01
Harvest (H)			<.01	<.01	<.01
TxB	0.20	0.25	0.25	0.44	0.12
TxF	0.53	0.15	0.01	0.80	0.35
TxH			0.45	0.60	0.91
RxF	<.01	<.01	0.01	0.51	0.04
BxH			<.01	<.01	<.01
FxH			<.01	<.01	<.01
TxBxF	0.29	0.56	0.19	0.77	0.01
TxBxH			0.41	0.60	0.14
TxFxH			0.03	0.80	0.23
BxFxH			0.19	0.28	<.01
TxBxFxH			0.16	0.52	0.91

Effect	pH	P (ppm)	Ca (ppm)	
Biochar treatment (T)		0.09	0.11	0.16
Biochar rate (B)		<.01	<.01	<.01
Fertilizer rate (F)		<.01	0.61	<.01
TxB		0.57	0.08	0.30
TxF		0.12	0.15	0.25
BxF		<.01	<.01	0.01
TxBxF		0.99	0.18	0.52

Draft