Plants grown in the University of Montana's venerable Memorial Greenhouse as part of a biochar study.

Biochar effects on the nursery propagation of 4 northern Rocky Mountain native plant species

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ABSTRACT

Biochar has emerged as a promising potential amendment of soilless nursery media for plant propagation. With this greenhouse study we used biochar to displace standard soilless nursery media at 4 rates (0, 15, 30, and 45% [v:v]) and then examined media chemistry, irrigation frequency, and the growth of 4 northern Rocky Mountain native plant species: *Clarkia pulchella* Pursh (Onagraceae), *Festuca idahoensis* Elmer (Poaceae), *Gaillardia aristata* Pursh (Asteraceae), and *Pinus ponderosa* Lawson & C. Lawson (Pinaceae). For all 4 species, media electrical conductivity (EC) showed little variation and was unaffected by biochar treatment. *Pinus* and *Gaillardia* media pH was lowered by all biochar levels, but the magnitude of difference was minimal. For *Pinus*, *Gaillardia*, and *Clarkia*, final seedling shoot, root, and total biomass production for all biochar treatments were similar. Only *Festuca* experienced a negative biochar effect regardless of biochar rate; root biomass was reduced, which was also reflected in lower total biomass and lower root-to-shoot ratios. Notably, adding biochar reduced watering frequency for all 4 species, with the magnitude of reduction directly proportional to biochar content. In the most extreme case (*Festuca*), controls required 55% more frequent irrigation than the 45% biochar treatment. This research shows that for 3 of the 4 species studied, soilless nursery media can be amended by up to 45% biochar content with minimal costs to plant growth and a reduction in watering frequency.

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KEY WORDS

Clarkia pulchella, *Gaillardia aristata*, *Pinus ponderosa*, *Festuca idahoensis*, soilless nursery media, pH, electrical conductivity, irrigation, Asteraceae, Onagraceae, Pinaceae, Poaceae

NOMENCLATURE

USDA NRCS (2017)

Photos by Clarice P Matt except as noted otherwise

CONVERSIONS
1 mm = 0.04 in
1 cm = 0.4 in
1 ml = 0.034 fl oz
(°C × 1.8) + 32 = °F
1 g = 0.035 oz

Native plant revegetation has become an important component of ecosystem restoration projects, requiring an increasing supply of nursery seedlings to meet a growing demand. Concurrently, a focus on the sustainability of native plant propagation practices has developed, including a movement among some container nursery practitioners to identify suitable alternatives to typical soilless growing media components (Landis and Morgan 2009). Biochar has emerged as one such material that shows promise as a partial replacement of those media components, including peat (Caron and Rochefort 2013; Steiner and Harttung 2014; Méndez and others 2015; Di Lonardo and others 2017), perlite (Northup 2013), and vermiculite (Dumroese and others 2011; Headlee and others 2014). Biochar is a co-product of the bioenergy pyrolysis process during which any organic material (feedstock) is heated in a zero- to low-oxygen environment, resulting in a stable, carbon-rich material. In nursery plant propagation, biochar may be a beneficial amendment for standard growing media, potentially bringing benefits to plant productivity, reducing reliance on non-renewable media components, and contributing carbon sequestration benefits to restoration projects (Dumroese and others 2011).

Biochar's potential to enhance nutrient dynamics in native plant propagation represents a much-needed area of research. Past studies have shown a range of plant growth responses (–29% to +324%) resulting from a wide range of biochar application rates and a multitude of feedstock types (Glaser and others 2002). Studies of biochar in soilless substrates are less common than studies conducted in mineral soils, but recent studies reported benefits of biochar to plant performance through nutrient retention (Altland and Locke 2012), plant growth (Graber and others 2010), and resistance to pathogenic fungi (Zwart and Kim 2012).

We conducted a greenhouse study to examine the effects of biochar on the growth of 4 northern Rocky Mountain native plant species. The species represent 4 plant life forms: 1) an annual herbaceous forb, *Clarkia pulchella* Pursh (Onagraceae; pinkfairies); 2) a perennial graminoid, *Festuca idahoensis* Elmer (Poaceae; Idaho fescue); 3) a perennial herbaceous forb, *Gaillardia aristata* Pursh (Asteraceae; blanketflower); and 4) a long-lived woody tree species, *Pinus ponderosa* Lawson & C. Lawson (Pinaceae; ponderosa pine). We investigated effects on plant growth and media properties by assessing whether biochar amendments could improve standard nursery media properties and would lead to enhanced seedling production. The study employed an experimental design to address the following research questions:

1. Does biochar affect the electrical conductivity (EC) and pH properties of the nursery media? If so, does the effect vary by biochar content?
2. Does biochar affect seedling growth? If so, does the effect vary by biochar content?

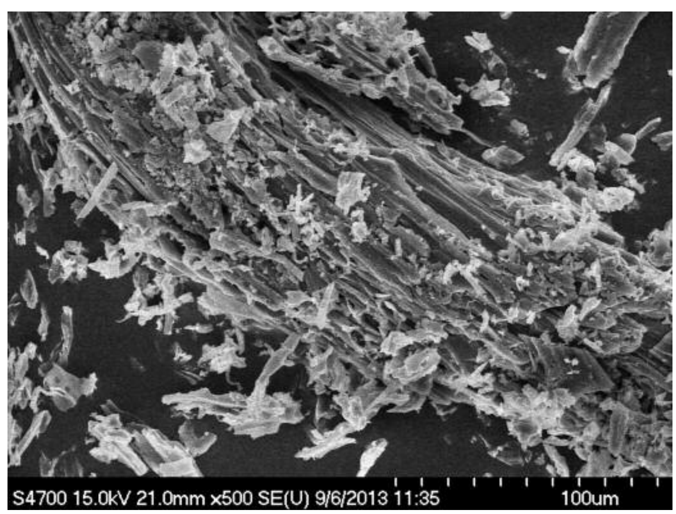
The purpose of the study was to inform nursery managers of the potential utility of biochar in the propagation of these 4 native plant species: namely, we sought to determine whether biochar displacement of more commonly used soilless media products would result in gains or losses in seedling biomass production.

MATERIALS AND METHODS

To answer our research questions, we used a multifactorial block design with 4 rates of biochar amendment, 4 species, 4 sample dates, and 4 replications. We manually incorporated CQuest biochar (Dynamotive Energy Systems Corporation; Richmond, British Columbia, Canada) into a 3:1:1 (v:v:v) ratio of peat, perlite, and vermiculite growing medium (a formulation commonly used by regional nurseries) at rates of 0, 15, 30, and 45% by volume. Four batches (replicates) of each rate were mixed in sufficient quantity to provide enough treated media to fill a complete replicate for all 4 species. Biochar was produced from the pyrolysis (400–500 °C) of agricultural and forestry residues (cellulosic biomass having < 10% moisture by mass and 1–2 mm particle size). The biochar was 74% carbon with a C:N ratio of 199, and most particles (88%) were < 0.5 mm (for more chemical and physical details on the biochar, see Dumroese and others 2011).

We used Ray Leach Super Cell containers (Stuewe & Sons, Tangent, Oregon). Each tapered container had a top diameter of 3.8 cm, depth of 21 cm, and a volume of 164 ml; a tray holds 98 containers. We selected this particular container because it is commonly used in native plant nurseries, it is easily handled, and the individual containers can be rearranged within or between trays as needed.

Our study design included 4 sample dates to assess treatment responses throughout the growing season. Because of the



Electron microscan image of Dynamotive's CQuest biochar at 200× magnification.

anticipated small size of seedlings early in the experiment, we chose to composite 3 seedlings of each species within each replication at each sample date. Therefore, we filled 768 containers (4 biochar rates × 4 species × 4 sample dates × 3 seedlings per composite × 4 replications). Containers for each replicate were filled in thirds to a uniform density. At onset of the experiment and to facilitate subsequent handling and fertigation (described below), an entire replicate (which included the 4 sample dates x 3 seedlings per sample date; 12 seedlings total) of each treatment for each species was loaded into a single tray. Thus, we employed 64 trays total (4 media × 4 replicates × 4 species).

Seeds, acquired from local vendors to ensure locally adapted seed sources (*Festuca*, Westland Seed, Ronan, Montana; *Gaillardia* and *Clarkia,* Native Ideals Seed Farm, Arlee, Montana; *Pinus,* Inland Empire Tree Improvement Cooperative's seed orchard, Missoula, Montana), were sown directly into prepared treatment containers in late April and early May. Prior to sowing, we surface disinfested *Pinus* seeds in an 8:1 (v:v) tap water:bleach (5.25% sodium hypochlorite) solution for 8 min, placed in a running water soak for 48 h, and subsequently stratified 45 d at 3 °C . To ensure a sufficient number of complete experimental units, multiple seeds were sown in each container. Germinants were thinned to one seedling per container. Additionally, 25 extra containers of each species–treatment combination, as well as 2 extra "filler" replicates, were sown as mortality substitutes to minimize the need for transplanting and to ensure seedlings in 4 complete replicates.

Sown containers were placed inside a greenhouse at the University of Montana (Missoula; 46.85863, –113.98391) and seedlings were grown during the course of one growing season: May to December. Daytime temperatures were maintained between 21 and 25 °C until the end of the active growth phase (end of August) when temperatures were reduced to promote hardening off. Seedlings were fertilized once each week in conjunction with irrigation by way of siphon injection (fertigation). We used General Hydroponics' (Santa Rosa, California) FloraDuo, a water soluble, two-part, complete (including

Blocks of *Pinus ponderosa*, *Gaillardia aristata*, *Festuca idahoensis*, and *Clarkia pulchella* seedlings at an early-season fertigation event.

micronutrients) nutrient system (Part A: 5-0-6; Part B: 1-5-4) in a 1:1 ratio. Fertilizer concentrations were determined to achieve an application rate of 150 ppm of nitrogen (N) during each fertigation event. Once the *Pinus* formed visible terminal buds in early September, we decreased the N rate to 75 ppm for the *Pinus* and *Gaillardia*, a commonly used practice to encourage hardening (Landis and others 1989). Seedlings were fertigated (once each week) or irrigated (as often as necessary) based on gravimetric water contents (Dumroese and others 2015). When the mass of the water within containers within each replicate reached 75% of the total mass of water held by each media (that is, container capacity mass), seedlings were irrigated or fertigated.

Data Collection

Four destructive harvests for each plant group (short-season species [*Clarkia* and *Festuca*] and long-season species [*Pinus* and *Gaillardia*]) were undertaken at either 3, 6, 9, and 12 wk or 8, 14, 20, and 26 wk, respectively. At each scheduled destructive harvest, we processed 3 composite seedlings and averaged measures for each replicate. Roots and shoots were separated, rinsed with deionized water, and dried 48 h in a forced-air drying oven at 70 °C. Dry mass was determined using an analytical balance (Ohaus Explorer EO1640, Pinebrook, New Jersey).

Table 1 lists the growth measurements tested for treatment-related differences per species. For all 4 species, we separately recorded shoot biomass and root biomass, then calculated total biomass and root-to-shoot ratio (R:S) from those measurements. Additional growth metrics beyond those differed by species due to inherent differences in growth forms.

We measured EC and pH of the final harvest media samples according to the 2:1 method described by Landis and Dumroese (2006). Once the leachate was obtained for each sample, EC and pH readings were determined using the Fieldscout Direct Soil EC Probe (Spectrum Technologies model # 2265FS, Aurora, Illinois) and a pHep 4 temperature-adjusted pH meter (Hanna Instruments model # HI 98127, Woonsocket, Rhode Island), respectively. EC and pH meters were recalibrated after the completion of each replicate and rinsed thoroughly with deionized water to maintain quality control and to prevent contamination between samples.

Data Analysis

Growth data gathered at each destructive sampling harvest was used to create total seedling biomass aggradation curves for visual assessment of treatment-related differences in growth rates for each species over time. Watering frequency was recorded to identify any treatment-related trends. By final harvest, we expected that biochar would result in enhanced media chemical properties and measures of plant growth, compared with the standard biochar-free media mix. Using statistical software R (R Core Team 2013), we tested the following hypotheses at final harvest:

1. Biochar amendment will alter nursery media electrical conductivity (EC) and pH; and
2. Native plant species grown in biochar-amended nursery media will produce significantly more dry biomass than those grown in the control treatments.

Wherever possible, we applied analysis of variance (ANOVA) *F*-tests to identify significant differences among treatments for all response variables ($\alpha = 0.05$), separately for each species, using transformed or untransformed data (as appropriate) on final harvest data. ANOVA *F*-tests are robust against variance heteroskedasticity when sample sizes are equal, as was the case here. The normality assumption was evaluated with the Shapiro-Wilk normality test (Shapiro and Wilk 1965) at $\alpha = 0.05$. We evaluated the assumption of equality of variances with Bartlett's test for homogeneity of variances (Snedecor and Cochran 1989) at $\alpha = 0.05$. For those response variables that did not meet the ANOVA model assumption of normality, we utilized Kruskal-Wallis non-parametric tests of stochastic dominance ($\alpha = 0.05$) in lieu of ANOVA to identify significant differences among treatments (Kruskal and Wallis 1952).

TABLE 1

Schedule of destructive sampling dates and growth parameters.

Species	Season length	Growth habit	Growth responses	Harvest dates
Clarkia pulchella	Short season	Annual herbaceous forb	Total biomass, shoot biomass, root biomass, seedling height, R:S	Wk 3, 6, 9, 12
Festuca idahoensis	Short season	Perennial graminoid	Total biomass, shoot biomass, root biomass, length of longest leaf, bunch diameter, R:S	Wk 3, 6, 9, 12
Gaillardia aristata	Long season	Perennial herbaceous forb	Total biomass, shoot biomass, root biomass, number of true leaves, R:S	Wk 8, 14, 20, 26
Pinus ponderosa	Long season	Long-lived woody tree	Total biomass, shoot biomass, root biomass, seedling height, R:S	Wk 8, 14, 20, 26

Notes: Harvest dates refer to the number of weeks following first fertigation event, which occurred 2 wk following sowing.

For *Clarkia*, untransformed root biomass, height, and media pH met both ANOVA assumptions. No transformations successfully resolved the issue of variance heteroskedasticity present for shoot biomass, total biomass, and R:S. No data transformation resolved either the normality or the unequal variances for media EC, so non-parametric Kruskal-Wallis tests were used to identify significant differences instead of ANOVA.

For *Festuca*, untransformed shoot biomass, longest leaf length, bunch diameter, media EC, and media pH met both ANOVA assumptions. Log transformations of root biomass, total biomass, and R:S successfully resolved the normality assumption. All response variable data—untransformed or log-transformed—met the equal variances assumption.

For *Gaillardia*, untransformed shoot biomass, root biomass, total biomass, leaf count, R:S, and media pH met the normality and equal variance assumptions. Log transformation of media EC successfully resolved the normality and equal variance assumptions.

For *Pinus*, untransformed shoot biomass, seedling height, and R:S met both model assumptions of normality and equal variances. No transformation successfully resolved variance heteroskedasticity for root biomass and total biomass. No transformation successfully resolved deviations from normality observed for media EC and media pH, so we employed Kruskal-Wallis non-parametric tests instead of ANOVA.

When the ANOVA tests indicated a significant treatment effect, we applied Tukey's Honestly Significantly Different (Tukey's HSD) multiple comparison post hoc tests (Tukey 1949) to further distinguish significant differences among all possible treatment comparisons ($\alpha = 0.05$). If significant differences were found for the non-parametric Kruskal-Wallis test, we used a post hoc Nemenyi test to identify which comparisons were significant ($\alpha = 0.05$).

RESULTS

Media Chemistry

We observed few biochar effects on media chemistry (Table 2). Media EC for all 4 species was unaffected by biochar level (all $P > 0.485$), and media pH for the short-season species, *Clarkia* and *Festuca*, was unaffected by biochar (both $P > 0.485$). For *Pinus*, we found weak but statistically significant evidence of lowered pH by biochar treatment at the 45% level ($P = 0.0262$) (no difference from the control at 15% and 30% biochar treatment levels). For *Gaillardia*, biochar at the 45% and 30% treatment levels resulted in lower pH ($P = 0.0033$) (no difference from the control at the 15% biochar level).

Growth Trajectories

For the short-season species, *Clarkia* and *Festuca*, growth trends were observed during the 12-wk period between germination and final harvest. For *Clarkia*, growth trajectories for each biochar treatment did not differ much from that of the control, particularly by the second harvest (Figure 1). The control exhibited greater total biomass initially, but by the final harvest this effect had vanished. One interesting trend is the apparent reduced biomass that occurred during early seedling establishment within the biochar treatments. At the first harvest, seedlings in all of the biochar treatments exhibited markedly less biomass when compared to the control. By the final harvest, however, mean total biomass for all treatments was comparable. For *Festuca*, the growth trajectories for all treatments were similar (Figure 1). Unlike *Clarkia*, *Festuca* biomass accumulation in the biochar treatments did not differ from the control. For the first 2 harvest dates, all biomass accumulation curves were comparable. For the last 2 harvest dates, biomass accumulation began to differentiate among treatments.

Growth trends for the longer-season species, *Gaillardia* and *Pinus*, were observed for a longer period of time: 26 wk. Growth trends for *Gaillardia* exhibited the greatest amount of treatment variation compared to the other species (Figure 1). The observed rate of biomass accumulation was most constant for the 30% biochar treatment; all other groups showed accumulation rates that slowed over time. *Pinus* exhibited the least amount of variation in overall mean total biomass accumulation, relative to the study's other 3 species (Figure 1). Growth trajectories for the control and the 45% biochar treatment were similar.

Seedling Attributes

We observed few effects on the growth of *Clarkia*, *Gaillardia*, and *Pinus* seedlings for any biochar level (Table 2). Specifically, biochar had no effect on shoot, root, or total biomass (Figure 2) (all $P > 0.13$); correspondingly, biochar had no effect on R:S (all $P > 0.17$), which averaged 0.55 for *Clarkia*, 3.29 for *Gaillardia*, and 1.15 for *Pinus*. *Gaillardia* leaf count ($P = 0.142$) and *Clarkia* shoot height ($P = 0.827$) were unaffected by biochar. For *Pinus*, all shoot heights were similar except for the 30% biochar treatment, which produced seedlings significantly ($P < 0.007$) taller (19.8 cm) than the control (16.6 cm), 15% biochar (16.1 cm), and 45% biochar (16.9 cm).

Festuca, however, responded to biochar treatment, primarily with reduced root biomass (Table 2). The control yielded significantly more root biomass ($\bar{x} = 4.10$ g) than did all 3 biochar treatment levels (all $P \leq 0.018$) (Figure 3). Correspondingly, the control yielded significantly more total seedling biomass ($\bar{x} = 6.40$ g) than did the 15% biochar ($\bar{x} = 4.46$ g, $P = 0.016$) and 30% biochar ($\bar{x} = 4.36$ g, $P = 0.010$) treatments (Figure 2). Similarly, R:S of the controls ($\bar{x} = 1.80$) was greater than the 30% biochar ($\bar{x} = 1.12$, $P = 0.017$) and 15% biochar ($\bar{x} = 1.20$, $P = 0.033$) treatments (Figure 3). Neither shoot biomass ($P = 0.217$) nor longest leaf length ($P = 0.311$) was affected by biochar treatment (Table 2), and just the 15% biochar treatment produced modestly narrower bunch diameters ($\bar{x} = 29.41$ mm, $P = 0.040$) than the control ($\bar{x} = 31.93$ mm).

TABLE 2

Final response variable means (standard errors).

Species	Biochar (%)	Biomass (g) Root	Biomass (g) Shoot	Biomass (g) Total	Root-to-shoot	Height (cm)		Media pH	Media Electrical conductivity (mS/cm)
Clarkia pulchella	0	2.65 (0.19)	4.41 (0.43)	7.05 (0.47)	0.62 (0.09)	38.3 (0.7)		7.25 (0.15)	0.28 (0.07)
	15	2.61 (0.17)	4.34 (0.28)	6.95 (0.45)	0.60 (0.01)	36.3 (2.1)		7.30 (0.09)	0.32 (0.08)
	30	2.60 (0.38)	4.47 (0.17)	7.35 (0.49)	0.54 (0.07)	36.0 (2.1)		7.40 (0.05)	0.29 (0.06)
	45	1.92 (0.09)	4.31 (0.05)	6.28 (0.05)	0.45 (0.03)	36.1 (2.6)		7.31 (0.05)	0.27 (0.01)
	P value	0.8	0.7	0.3	0.2	0.8		0.7	0.8[y]
						Bunch diameter (mm)	**Length of longest blade (cm)**		
Festuca idahoensis	0	4.10 (0.52)	2.30 (0.34)	6.40 (0.66)	1.80 (0.18)	31.9 (0.4)	18.2 (0.3)	7.25 (0.08)	0.38 (0.20)
	15	2.62 (0.17)	1.84 (0.16)	4.46 (0.14)	1.45 (0.16)	29.4 (0.3)	16.4 (0.4)	7.25 (0.04)	0.34 (0.04)
	30	2.30 (0.16)	2.06 (0.17)	4.36 (0.27)	1.12 (0.05)	30.2 (0.4)	18.2 (0.3)	7.25 (0.05)	0.35 (0.04)
	45	2.61 (0.18)	2.24 (0.22)	4.84 (0.27)	1.18 (0.10)	30.0 (0.4)	17.8 (0.4)	7.43 (0.01)	0.28 (0.38)
	P value	0.003[z]	0.2	0.008[z]	0.014[z]	0.047	0.3	0.2	0.9
						Final leaf count (n)			
Gaillardia aristata	0	3.51 (0.52)	1.16 (0.33)	4.67 (0.71)	3.10 (0.28)	37.6 (1.2)		7.79 (0.02)	0.28 (0.14)
	15	4.61 (0.47)	1.59 (0.09)	6.20 (0.62)	2.87 (0.28)	39.6 (2.3)		7.65 (0.05)	0.32 (0.23)
	30	5.56 (0.95)	1.57 (0.54)	7.13 (1.26)	3.55 (0.23)	58.6 (1.8)		7.49 (0.06)	0.37 (0.20)
	45	5.47 (1.17)	1.44 (0.46)	3.91 (1.62)	3.68 (0.37)	42.2 (1.8)		7.49 (0.06)	0.35 (0.37)
	P value	0.4	0.5	0.4	0.2	0.1		0.003	0.9[y]
						Height (cm)			
Pinus ponderosa	0	2.18 (0.31)	1.83 (0.39)	4.01 (0.51)	1.23 (0.21)	16.5 (0.5)		7.42 (0.02)	0.31 (0.13)
	15	2.18 (0.24)	1.97 (0.25)	1.15 (0.37)	1.11 (0.15)	16.1 (0.6)		7.24 (0.02)	0.29 (0.14)
	30	2.57 (0.19)	2.57 (0.38)	2.14 (0.51)	1.03 (0.17)	19.8 (0.9)		7.21 (0.01)	0.27 (0.06)
	45	2.24 (0.03)	2.24 (0.03)	4.04 (0.06)	1.24 (0.07)	16.9 (0.4)		7.20 (0.01)	0.28 (0.21)
	P value	0.4	0.2	0.2	0.2	0.007		0.026[y]	0.5[y]

[z] *P* values are for log transformed data and ANOVA tests.
[y] *P* values for Kruskal-Wallis tests.

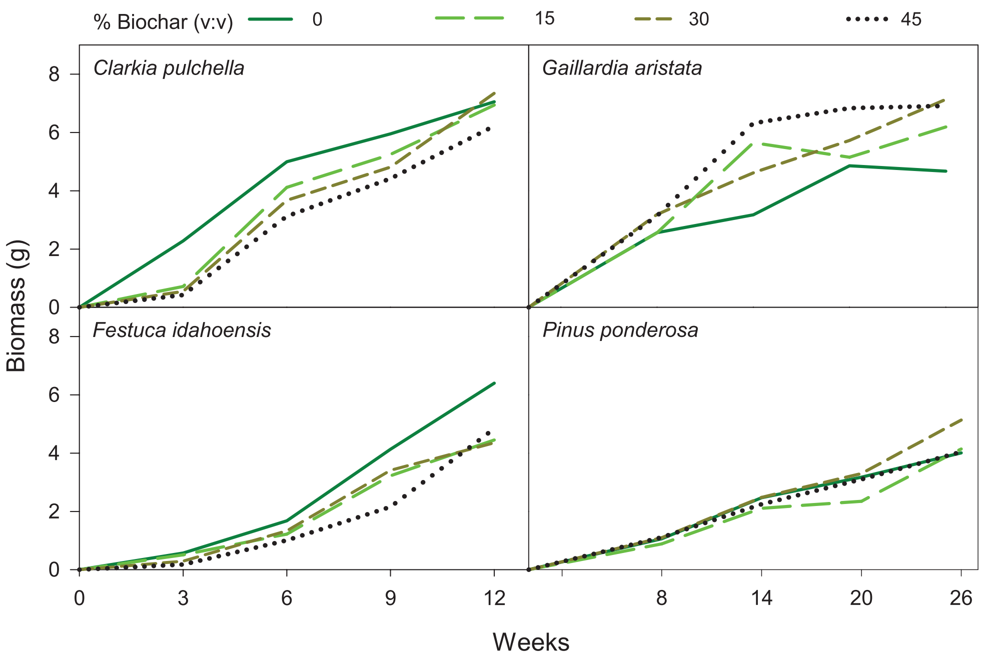


Figure 1. Mean total biomass accumulation (g) as a function of biochar treatment during 12 wk of growth for short-season *Clarkia pulchella* and *Festuca idahoensis* and during 26 wk of growth for long-season *Gaillardia aristata* and *Pinus ponderosa*.

Watering Frequency

Irrigation records indicate a common trend regarding the relation of biochar and watering frequency: For each species, biochar treatments resulted in reduced watering frequency (compared with the controls), with the magnitude of that reduction positively related to biochar level (Table 3). Compared to the 45% biochar treatment for all species combined, controls required 39% more frequent irrigation to maintain container capacity mass. The greatest difference in watering frequency was observed for *Festuca*, for which the control required nearly 55% more frequent irrigation than the 45% biochar treatment.

DISCUSSION

Media Electrical Conductivity (EC)

Substrate EC provides an estimate of the amount of fertilizer (measured as solubilized electrically charged ions) present in a growing substrate. For most plants, the recommended range of EC to allow for normal growth in established plants, using the 2:1 method, ranges from 0.76 to 1.25 millisiemens (mS). Values ranging from 0.26 to 0.75 mS are, however, considered suitable for seedlings (BC Ministry of Agriculture and Food 1999). Although our observed values (0.28–0.38 mS/cm) fell at the low end of that range, they would generally be considered adequate for seedling growth (Fisher and Argo 2005; Camberato and others 2009).

EC can increase (Vaughn and others 2013) or decrease (Steiner and Harttung 2014) in response to biochar additions, or—as was the case with our study—reveal no clear trend. Northup (2013) conducted a 16-wk greenhouse experiment using hardwood biochar of varying particle sizes and application rates (10% to 100% biochar by volume, in 10% increments) blended with peat to determine effects on media pH, EC, and physical properties and plant growth (species not specified). That study found that pH increased as biochar rate increased and particle size decreased but found no clear trend in media EC as a function of either biochar rate or particle size. We found a similar lack in EC trends from our 3 biochar treatments. Similarities in biochar rates, biochar particle size, media components, and study duration in this study offer a better opportunity for comparison to other biochar studies with shorter sampling times, duration, and substantially lower application rates. Interestingly, studies that reported increases in media EC did not report corresponding increases in plant growth (Vaughn and others 2013), and vice versa (Steiner and Harttung 2014).

Media pH

The primary effect of pH on plant growth is its role in nutrient availability. Most native plants tend to grow best at pH values ranging from 5.5 to 6.5 (Jacobs and others 2008), because the availability of micro- and macronutrients essential for normal plant growth is maximized. Yet, in our study the pH values ranged from 7.2 to 7.8 without any apparent negative effects on plant growth. One possible explanation may be that we applied sufficient quantities of supplemental nutrients. Studies indicate that biochar raises pH in soilless media (Tian and others 2012;

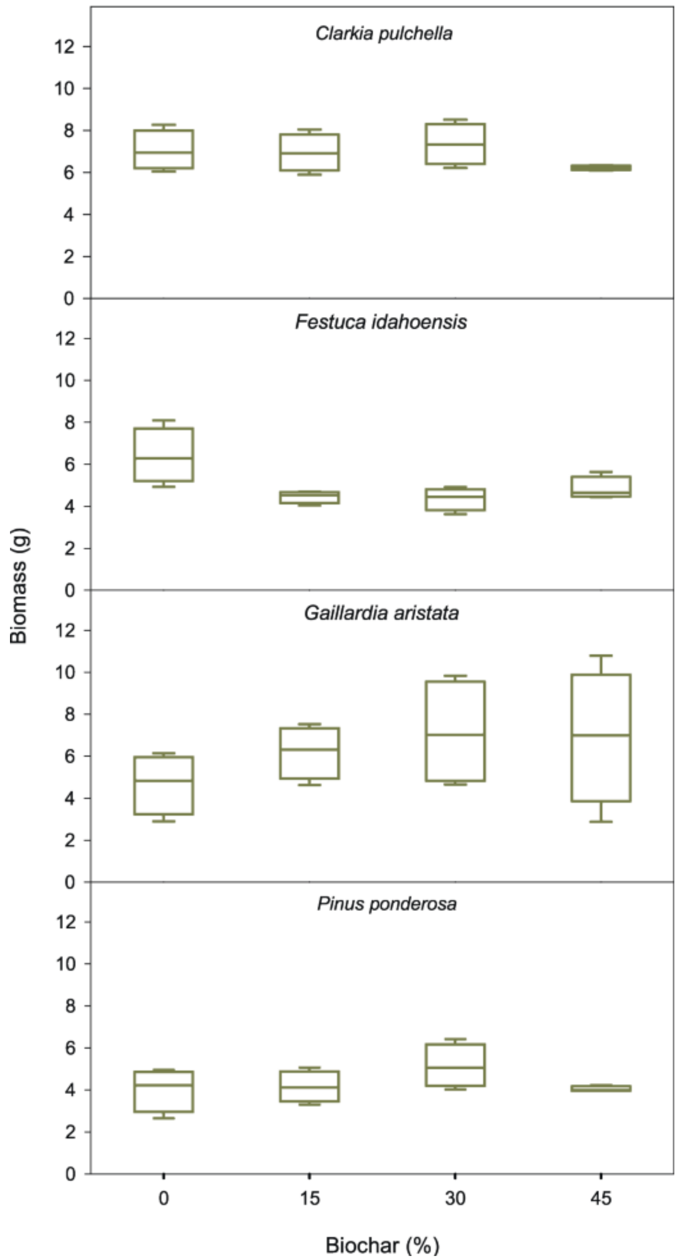


Figure 2. Mean total biomass (g) by biochar treatment after 12 wk for short-season *Clarkia pulchella* and *Festuca idahoensis* and after 26 wk for long-season *Gaillardia aristata* and *Pinus ponderosa*. Only *Festuca* was significantly affected by biochar, with total biomass reduced by biochar at the 15% and 30% (v:v) levels. Vertical boxes represent approximately 50% of the observations, and lines extending from each box are the upper and lower 25% of the distribution. The solid horizontal line in the center of each box is the median value.

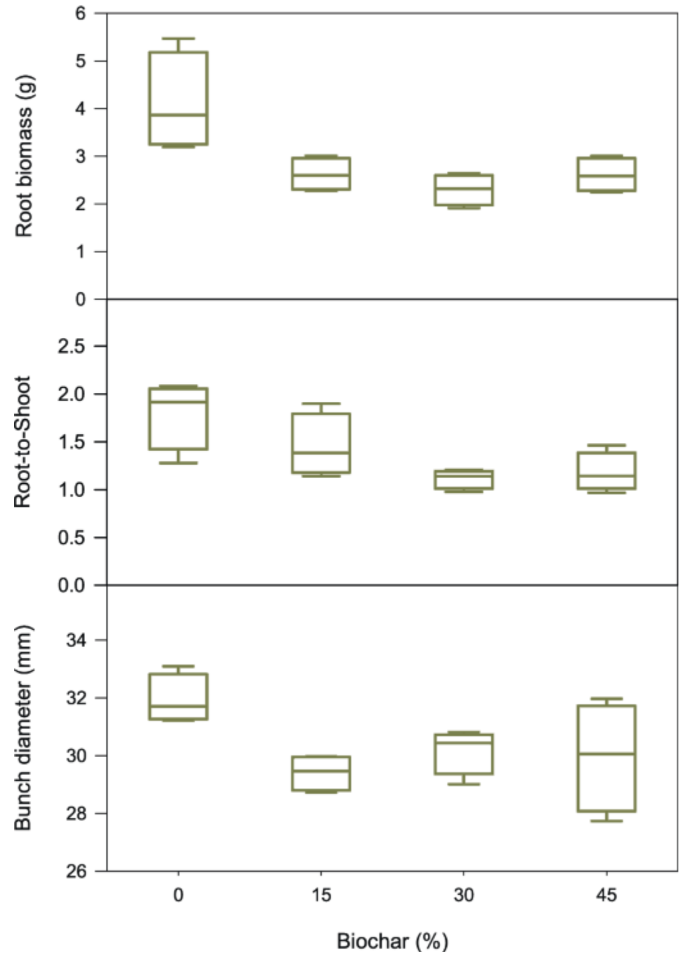


Figure 3. Festuca idahoensis growth responses by treatment. Compared to the control, addition of biochar significantly reduced root biomass (g) at any biochar level, root-shoot ratio (R:S) at the 15% and 30% biochar levels, and bunch diameter (mm) at the 15% biochar level. No statistically significant differences occur among the 3 biochar levels for those metrics. Vertical boxes represent approximately 50% of the observations, and lines extending from each box are the upper and lower 25% of the distribution. The solid horizontal line in the center of each box is the median value.

Vaughn and others 2013), allowing it to act as a suitable replacement for the lime necessary to maintain adequate pH values in peat-based growing media (Northup 2013; Steiner and Harttung 2014). In our study, however, biochar did not raise pH at any application level; to the contrary, some biochar levels produced a moderate but statistically significant lowering of pH for *Gaillardia* and *Pinus* (but we note that even in those cases, the difference in pH was slight). Possibly explaining the contrary trend in media pH was the more frequent irrigation in the control treatments and the relatively high pH of the irrigation water used in the greenhouse (7.6) that caused the pH of the control media to increase during the experiment.

Growth Trajectories

During early seedling establishment, we noted a lag in biomass accumulation for *Clarkia pulchella* for all 3 biochar treatments (see Figure 1). This trend likely resulted from the need for biochar to charge with sufficient nutrients prior to making them available for plant uptake. None of the other study species exhibited this lag in biomass accumulation during seedling establishment. The sampling timeline seemed to align especially well with the *Clarkia* development pattern and thereby enabled us to capture the charging effect; it is possible that biochar

TABLE 3

Number of irrigation events, as triggered by a 75% container capacity threshold.

		Biochar (%)		
Species	**Control**	**15**	**30**	**45**
Clarkia pulchella	38	36	32	27
Festuca idahoensis	31	26	21	20
Gaillardia aristata	53	51	44	41
Pinus ponderosa	49	44	39	37

Notes: For all 4 species, irrigation frequency was inversely related to biochar content.

charging occurred in the long-season species as well, but the sampling timeline was not sufficiently frequent to capture it.

The need for fresh biochar to charge is well known within the context of biochar application to field soils. Fresh biochars have low cation exchange capacity (CEC) values that increase over time as the surface of the biochar is exposed to air and water (Cheng and others 2006, 2008; Liang and others 2006). Biochar surface oxidation increases the overall negative charge thereby increasing CEC. The same is likely to occur in soilless media. Biochar charging could potentially be observed by tracking media EC over time in conjunction with observations of plant growth and plant tissue color. We anticipated that initial EC values would remain low and plants would exhibit suppressed growth and chlorotic plant tissue color that would begin to improve over time as exchange sites were charged with nutrients, freeing subsequent supplemental nutrients for plant uptake. But studies tracking the effect of biochar on media EC over time have found no clear trend as a function of biochar amendment (Kachenko 2011; Northup 2013). Lack of an observed biochar charging phenomenon may be explained by the sampling timelines, as we theorize may have been the case in our study. Perhaps biochar charging in soilless media occurs during a shorter time frame than in field soil because of the optimal water and nutrient conditions provided in the nursery.

Seedling Attributes

Our study found no notable biochar treatment effects on plant growth with the exception of *Festuca idahoensis*, for which biochar reduced root biomass and related metrics. Growth parameters were neither enhanced nor diminished by biochar in *Clarkia*, *Gaillardia*, and *Pinus*. The latter result is similar to other findings with crop and horticultural species (Vaughn and others 2013) but contrary to the positive correlations in seedling dry weight reported by others (Graber and others 2010; Tian and others 2012; Headlee and others 2014). Positive correlations in seedling biomass were not a result of enhanced seedling nutrition but rather from increased resist-

The annual forb *Clarkia pulchella* has many common names, including pinkfairies, wild Clarkia, deerhorn, and ragged robin. Photo by Terry Gray

Gaillardia aristata is a drought-tolerant perennial forb that produces numerous, large, showy flowers that support pollinators. Photo by Tara Luna ance to pathogenic fungi (Graber and others 2010; Zwart and Kim 2012). Biochar can decrease nutrient leaching in soilless media (Altland and Locke 2012), but positive plant growth responses have not correlated with increased plant tissue nutrients (Graber and others 2010) or electrical conductivity (Vaughn and others 2013).

Differences in biochar properties, application rates, and study species may explain inconsistent results, which further indicates the need to standardize study parameters in future biochar research in soilless media (Gundale and Deluca 2007; Mukome and others 2013). The properties of biochar vary according to feedstock and pyrolysis conditions (Downie and others 2009; Novak and others 2009; Rajkovich and others 2011). Feedstock largely influences inherent nutrient concentration and porosity, whereas pyrolysis conditions (temperature and heating time) influence C conversion to stable forms, pH, surface area, and CEC (Novak and others 2009).

Although we visually observed apparent trends indicating that biochar treatments yielded higher total seedling biomass than did the controls, those suggested differences were not statistically significant. We noted substantial within-treatment variation that may have obscured real differences in total biomass resulting from biochar amendment. We observed one such example in the *Gaillardia* 45% biochar treatment, in which mean total biomass was 6.9 g, but sample values spanned a broad range of 2.9 g to 10.8 g.

Watering Frequency

Our study revealed that biochar amendments resulted in less frequent irrigation despite no resulting differences in overall plant size (except for *Festuca*). Biochar alters various soil physical properties that affect soil aeration, water-holding capacity, and plant growth (Downie and others 2009). In field soils, biochar's high surface area can affect soil texture, aggregation, and total porosity, factors that can alter soil water retention (Glaser and others 2002; Downie and others 2009). Biochar amendment of soilless media should correspondingly alter media physical properties in a similar way (Northup 2013; Kaudal and others 2015). Biochar has a much smaller particle size than does perlite or vermiculite, which are common constituents of soilless media to improve drainage because their larger macropores are less capable of retaining water, particularly at low volumetric water contents.

As such, differences in particle size may explain the reduced irrigation frequency observed in the biochar treatments. That finding suggests that biochar could be used to reduce the water use and labor costs associated with irrigation in container nurseries. Furthermore, less-frequent irrigation suggests that seedlings grown in biochar-amended media could retain more water directly around the root zone after outplanting, giving those seedlings a distinct advantage during the crucial establishment stage following outplanting (Ritchie and others 2010).

Implications for Restoration

From a restoration standpoint, the ultimate test of success for a nursery seedling grown in biochar-amended soilless media is whether increased seedling establishment and growth occurs after outplanting. The advantages of biochar amendments to suboptimal field soils are well documented: Biochar enhances water-holding capacity (Iswaran and others 1980) and nutrient dynamics (Lehmann and others 2003a,b). Therefore, logic suggests that similar advantages may exist when seedlings grown in biochar-amended soilless media are outplanted. In that scenario, seedlings are no longer subject to optimal temperature, water, and fertilizer regimes but are instead subjected to ambient conditions and field soil properties, including nutrient and water limitations.

Soil moisture and its effect on nutrient uptake and translocation (for example, Helenius and others 2002) greatly influence plant survival and growth. Root systems of outplanted seedlings must be able to acquire sufficient water from the soil to meet shoot transpiration requirements (Ritchie and others 2010). In newly outplanted seedlings, water stress from insufficient soil moisture can reduce growth and increase mortality (Ritchie and others 2010). Rehydrating and increasing the amount of water retained in the root plugs of outplanted seedlings using root dips is common during the outplanting process; superabsorbent hydrogel can also be used to increase water retained in the seedling plug. One trial implementing this technique found lower seedling mortality 5 mo after outplanting, which was attributed to increased soil moisture or contact between the root plug and field soil (Thomas 2008). Enhanced water and nutrient status from biochar present in seedling plugs has potential to translate into increased survivability of outplanted seedlings, particularly within dry montane habitats that are common in the northern Rocky Mountains. Outplanting studies that investigate the potential advantage of biochar amendment on seedling establishment and survival are necessary. If positive relationships are found, that outcome would seemingly add value to seedlings propagated in biochar-based media and could thereby spur the market for biochar.

A significant diversity of biochars and growing media has led to varying effects on plant growth and media properties, posing a challenge to inferring their meanings for other biochars, plant species, and landscapes. We caution that extrapolating biochar effects found in this study to other native plant species and (or) to other growing media should be limited. Researchers expanding on studies investigating biochar application in container nursery native plant propagation can infer similar growth responses in native species within the same growth habit (that is, annual forb, perennial forb, long-lived tree, perennial graminoid). We advise that plant growth and studies be expanded to the range of species and site conditions for which native plants are employed in restoration projects.

CONCLUSIONS

To our knowledge, this study is the first to investigate the effects of biochar amendment on the nursery-propagated growth of an annual forb, a perennial forb, a perennial graminoid, and a long-lived woody tree species native to the northern Rocky Mountains. A diversity of biochar types exists, with likely diverse chemical and physical properties and possible plant growth effects. For the biochar used in this study, we found that for 3 of 4 plant species, biochar can displace up to 45% of standard peat, perlite, and vermiculite growing media without compromising the quality of propagated seedlings. With the exception of *Festuca*, biochar amendment had little effect on plant growth or media EC and pH. Biochar at all treatment levels produced EC and pH values that allowed for normal plant growth, despite being outside optimal ranges recommended for native plant species. Biochar reduced water use and irrigation frequency, with the magnitude of that reduction positively related to biochar level.

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