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1 **AN ASSESSMENT OF BIOCHAR AMENDED SOILLESS MEDIA FOR**
2 **NURSERY PROPAGATION OF NORTHERN ROCKY MOUNTAIN**
3 **NATIVE PLANTS**

4 By:

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6 B.A., University of Montana, Missoula, Montana, 2008

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27 **An assessment of biochar-amended soilless media for nursery propagation**
28 **of northern Rocky Mountain native plants**

29 Chairperson: Dr. Christopher R. Keyes

30 **Abstract**

31 A study was conducted to better inform nursery practitioners of the potential benefits of biochar
32 application in nursery media for native plant propagation. Biochar – a carbon-rich, recalcitrant
33 charred organic co-product of the bioenergy pyrolysis process – has emerged as a promising
34 potential replacement for peat and perlite in nursery seedling propagation. A strong conceptual
35 basis exists for biochar as a nursery media amendment, but empirical data on biochar-based plant
36 propagation is scarce. This greenhouse study examined the effects of biochar displacement of
37 standard soilless nursery media at rates of 0%, 15%, 30%, and 45% (percent volume
38 composition) on propagation of four western Montana native plant species: deerhorn clarkia
39 (*Clarkia pulchella* Pursh.), common blanketflower (*Gaillardia aristata* Pursh.), ponderosa pine
40 (*Pinus ponderosa* Doug.), and Idaho fescue (*Festuca idahoensis* Elmer). Biochar at any level
41 generally resulted in few differences in plant growth or media chemistry. Seedling biomass
42 production with biochar treatment was either equivalent to the standard media (control), or in the
43 case of *Festuca*, was slightly less. Exceptions include the final seedling height of the *Pinus* in
44 which the 30% biochar treatment grew significantly taller seedlings than the other treatment
45 groups. With regard to media chemistry, measured as pH and EC, little variation existed between
46 treatments in any of the study species. The *Pinus* and *Gaillardia* un-amended substrate had
47 significantly higher mean pH than the other biochar treatments, but the overall range of pH
48 values was small (< 1 pH units), and did not result in negative effects on plant growth. All
49 measures of plant growth for *Festuca*, except longest leaf length, resulted in significantly lower
50 measures for all the biochar treatment levels. Although few benefits of biochar incorporation
51 were identified, this research shows that biochar can suitably displace up to 45% standard peat,
52 perlite, and vermiculite mix without any drop in plant biomass growth for three of the study's
53 four species.

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Executive Summary

Our research aims to address a presently novel area of biochar research. The amount of current biochar knowledge that is applicable within the context of native plant container nursery seedling production is sparse. We conducted a greenhouse study to examine the effects of biochar on the growth of four northern Rocky Mountain native plant species: deerhorn clarkia (*Clarkia pulchella* Pursh.), common blanketflower (*Gaillardia aristata* Pursh.), ponderosa pine (*Pinus ponderosa* Doug.), and Idaho fescue (*Festuca idahoensis* Elmer). All four species were propagated from seed with biochar amending and displacing standard (3:1:1 peat, perlite and vermiculite v:v:v) soilless nursery media at rates of 0%, 15%, 30%, and 45% (percent volume composition). We investigated effects on plant growth and chemical substrate properties by assessing whether biochar amendments could: (1) improve standard nursery media properties leading to enhanced seedling productivity, and (2) amend or replace other commonly used nursery media products (such as non-renewable sphagnum peat) in the propagation of northern Rocky Mountain native plants. The goal of the study was to better inform nursery practitioners of the potential for biochar application in nursery media for native plant propagation.

Background

An increased emphasis on sustainability and environmentally sound uses of natural resources have led to many innovative efforts to minimize carbon footprints and negative impact to the environment. Incorporating climate change mitigation strategies into routine practices is one such approach to promote sustainability. In the realm of native plant restoration, the sustainability of certain nursery seedling propagation practices has come into question. Among these is the use of non-sustainable components (peat, perlite and vermiculite) in standard soilless growing mediums. Peat-based growing substrates are not necessarily nonrenewable, but they are

considered non-sustainable. Peatland ecosystems are important in terms of biodiversity, water filtration and as substantial C sinks. The problem with horticultural peat moss is the destruction of peat bog ecosystems to meet the global demand of peat for horticultural purposes (Caron and Rochefort 2013). Researchers are investigating the potential to replace peat and other commonly used nursery materials with more sustainable options (Dombrowky et al. 2013). Biochar is one material showing promise as a replacement for peat (Tian et al. 2012, Caron and Rochefort 2013, Steiner and Harttung 2014), perlite (Northup 2013) and vermiculite (Dumroese et al. 2011, Haard et al. 2009). Biochar as a means of promoting sustainability in the nursery, in conjunction with the benefits of biochar amendments to suboptimal field soils, has piqued the interest of potential biochar utilization in container nurseries.

The opportunity to investigate the potential for native plant propagation for restoration using biochar, while simultaneously sequestering carbon at no additional cost to production nurseries, stands to alter the sustainability of current propagation practices (Caron and Rochefort 2013). Our target audience spans a diverse field of professionals from biochar researchers, to restoration ecologists, to production nursery practitioners. The subsequent content is meant to set the stage so that every individual may find some component of our biochar for native plant propagation research useful. We hope to achieve this goal by providing information outlining the following: What biochar is; the conceptual basis for biochar application in the nursery; native plant propagation in soilless growing mediums; how biochar can address climate change; and information on the species selected for our study.

Biochar Defined

Biochar is known by many names in the scientific literature. It is synonymous with black carbon, pyrogenic carbon and charcoal. Biochar is a co-product of the bioenergy pyrolysis

process during which biomass is heated in low-oxygen to zero-oxygen environments resulting in carbon-rich, recalcitrant charred organic matter. All biochar is not created equally; its characteristics are dependent on pyrolysis conditions and feedstock sources (Downie et al. 2009). Most biochars share, however, key physical and chemical characteristics, which have been attributed with their benefit in field soil application, including a highly aromatic structure (Liang et al. 2006, Schmidt and Noack 2000), recalcitrance, alkalization effects on soil pH, high surface area, and a highly porous nature.

The conceptual basis for the application of biochar to field soils is supported by fire science and the existence of the Amazonian Anthrosols of South America (also known as Amazonian Dark Earths (ADEs) or *Terra Preta*; Lehmann 2007). Extensive research efforts on the functioning of *Terra Preta* soils have identified biochar additions as the primary mechanism explaining the enhanced, sustained fertility and carrying capacities of these soils. They are the foundation of a resurgence of interest in charcoal application as a soil amendment and its potential economic and environmental value to modern society (Van Zwieten et al. 2010).

Biochar and Soils

The benefits of biochar in mineral soils are well documented. Biochar amendments to field soils are shown to fundamentally enhance soil function. Amended soils often exhibit alterations to essential soil properties and conditions that determine, directly and indirectly, the fertility status of these soils. Strong evidence indicates that nutrient dynamics in soil can be significantly influenced by biochar (Lehmann 2003a, b). Soil fertility — and other factors greatly influencing soil fertility such as cation exchange capacity (CEC) (DeLuca and Aplet 2008, Liang et al. 2006), soil moisture, fertilizer retention, and the immobilization of toxic elements/compounds — responds positively to biochar additions (Atkinson et al. 2010).

Improvement of essential soil functions with biochar amendments occur during the long term (Lehmann, 2009). As a soil additive in conjunction with fertilizer, biochar improves: (1) nutrient availability (reduced leaching/increased retention) (Steiner et al., 2008), (2) plant productivity (Chan et al. 2007, Laird et al., 2010, Steiner et al. 2007, Yeobah et al. 2009), (3) soil tilth (Glaser et al. 2002), and (4) fertilizer use efficiency (Chan et al. 2007).

A beneficial reduction in nutrient leaching has been observed in a multitude of biochar studies in mineral soils (Ding et al. 2010; Knowles et al. 2011). The ability of biochar to reduce nutrient leaching is largely attributed to the charge and surface area properties of biochar. The increased nutrient retention and reduced leaching create obvious benefits to plant yield by increasing the bioavailability of essential nutrients, particularly in the presence of added nutrients (Atkinson et al. 2010). Reports suggest positive correlations between plant productivity and biochar additions, with results varying depending on the quantity of biochar added (Atkinson et al. 2010). As such, biochar amendments to soilless substrates show potential to produce similar results increasing plant productivity by reducing nutrient leaching and subsequently increasing nutrient use efficiency (Steiner et al. 2008). Biochar effects on soil pH also contributed to observed plant responses. Biochar additions to acidic field soils increase the pH of the soil solution, ultimately enhancing solubility of beneficial ions. This increase in the soil solution pH in turn affects nutrient availability, ultimately shifting the soil status closer to the optimal pH range for nutrient uptake by plants (Gundale and DeLuca 2007, Liang et al. 2006, Steiner et al. 2008). Optimal pH values range depending on the nutrient in question. The solubility of macronutrients needed in large amounts, such as nitrogen (N), phosphorus (P) and potassium (K) are particularly important for maximizing plant growth. But, managing the pH of growing media for a single nutrient will adversely affect plant growth by limiting the availability of other

essential macro and micro nutrients. For example, the optimal range for pH that maximizes the availability of N, P and K is about 6.5 to 7.5, 5 to 6, and 6 to 6.5, respectively (Figure 1). To ensure adequate levels of all essential mineral nutrients, media pH must be maintained between 5.5 and 6.5. It remains to be seen whether or not amending soilless growing substrates with biochar will garner the same beneficial effect on pH observed in biochar-amended field mineral soils. Alternative explanations for positive plant responses to biochar application unrelated to plant nutrition include toxin immobilization (Wardle et al. 1998), improved soil physical properties (Iswaran et al. 1980), or reduced soil strength (Chan et al. 2007).

Biochar and Climate Change

Science published an article by Pacala and Socolow (2004) titled, “Stabilization Wedges: Solving the Climate Problem for the Next 50 Years with Current Technologies,” in which they introduced the stabilization wedge theory that proposed atmospheric CO₂ concentrations must be limited to 500 ppm to avoid the most damaging climate change effects. This requires society to stabilize current CO₂ emissions leveling the current, “business as usual” (BAU) trajectory to 7 GtC/year for the next 50 years. They idealized the 50 year emissions reductions as a perfect triangle bordered by the BAU trajectory above and the stabilization trajectory below (see Pacala and Socolow 2004 for details). The triangle was further segmented into seven equal wedges, each representing a current mitigation strategy that can successfully sequester 1Gt C from the atmosphere during the next half century.

Biosequestration, also known as biological sequestration, is the process of sequestering carbon in living plant biomass, soil, and organic matter or in aquatic ecosystems by biological processes (www.biochar-international.org). It is useful to consider biosequestration from a carbon management perspective that can be approached using three strategies: 1) increase the

amount of carbon sequestered in plant biomass (phytosequestration); 2) actively sustainably manage the existing soil organic carbon (SOC) pool; or 3) increase the total SOC pool. Utilizing biochar in nursery production, land remediation and reclamation, as well as in agricultural production can contribute to the third biosequestration strategy, and feasibly account for one of the seven required mitigation wedges.

The conceptual basis for biosequestration and charcoal's ability to enhance soil fertility in the long term is founded in the existence of the Amazonian Anthrosols of South America (Lehmann 2007). The stability of ancient char additions to those soils indicates pyrogenic carbon remains stable for significant timescales, and therefore, can sequester significant amounts of carbon in soils. In the case of Amazonian dark earths, carbon dating of the char present indicates the potential for biochar to remain in the soil organic carbon pool for thousands of years.

Biosequestration is particularly feasible in the Rocky Mountain region, where much of the landscape is comprised of fire-dependent ecosystems. Fire science research has long indicated the importance of charcoal to the structure and functioning of these systems (DeLuca and Aplet 2008, Wardle et al. 1998). Fire-derived charcoal has been attributed as a significant component in post-fire ecosystem rejuvenation by enhancing N mineralization, nitrification, and minimizing allelopathy (DeLuca et al. 2002, Wardle et al. 1998). Scientific evidence suggests that biochar additions to forest soils will remain stable during the long term, and stand to benefit the structure and functioning of forest soils. As a result, incorporating biochar into native plant restoration in the northern Rocky Mountain region outplanting process provides a conceivable means of improving fertilizer use efficiency in production nurseries (Altland and Locke 2012), improving the structure and functioning of forest soils (Page-Dumroese et al. 2009), while quantifiably sequestering carbon in the long term (Laird 2008. Lal 2004).

pH, EC and soilless media

Native plant propagation for restoration in container nurseries is a common practice. Seedlings can be grown in containers in a variety of propagation environments where growth limiting factors, such as temperature, water, and fertilizer are controlled. Controlling growth limiting factors is an absolute necessity so that high quality seedlings are produced.

Growing media are arguably the most important part of the overall growing process in container nursery plant production. Soilless growing media provide anchoring material for developing roots and act as a reservoir of nutrients and water. Soilless growing media are composed of different organic and inorganic components such as peat, perlite, vermiculite, coir, rockwool, and bark blended together to create a growing environment with good aeration, nutrient supply, and plant available water. Soilless growing media with optimal physical and chemical characteristics promote plant growth and reduce the time necessary to produce saleable nursery crops. Physical properties of soilless growing media include water holding capacity (WHC), aeration, porosity, and bulk density. Chemical properties of growing media include fertility, pH, and cation exchange capacity (CEC). Soilless growing media are widely implemented in current production nursery practice as they pose several advantages compared with container soil-based media.

Container nursery production opts to use soilless growing media because of the poor aeration, soil pests and pathogens and weight limitations of soil-based media. Alternative substrate components exist, (such as coir, bark, and charcoal) but are rarely used on a large scale. Peat moss is by far the most commonly incorporated organic component of soilless growing media because it is readily available and creates an optimal, reliable growing environment with regard to physical and chemical substrate properties (nutrient levels, pH, WHC, porosity, and

CEC); it is, however, an expensive and non-renewable resource. Peat is commonly mixed with other aeration-improving materials such as perlite, vermiculite, and pumice. One disadvantage of soilless media is that continuous nutrient supplements must be managed in a container nursery setting, as some essential elements necessary for optimal plant growth are severely deficient or completely lacking from soilless media.

Managing important physical and chemical properties of growing medium is of vital importance because of the direct impacts these properties pose to providing optimal plant nutrition. Monitoring temporal variations in pH and EC enables nursery practitioners to play an active role in establishing, maintaining, and remedying adequate plant growth conditions. Routine monitoring of pH and EC is done without expensive, specialized equipment or facilities, and provides nursery practitioners with relevant information about growing conditions. Decent equipment for determining pH and EC can be obtained at a minimal cost to production nurseries, making pH and EC practical for small-scale and production nurseries.

The pH of the growing substrate is a measure of relative acidity or alkalinity. Values for pH range from 0 to 14; those above 7 are alkaline and those below 7 are acidic. Nutrient availability is largely determined by the pH of a growing substrate, and is primarily influenced by the effect of H^+ ions on the exchange complex. As such, pH directly influences the solubility, and therefore bioavailability, of various nutrient elements. Solubility of essential elements is important as plant roots are only capable of taking up nutrients that are dissolved in the substrate solution. Substrate pH that is neither too high nor too low is critical for optimizing the bioavailability of essential plant nutrients and avoiding micronutrient toxicity and deficiency.

In general, macronutrients tend to be less available at low pH, and micronutrients tend to be less available at high pH. The solubility of essential elements like manganese, zinc, iron and

boron increase in acidic substrate conditions. Conversely, concentrations of these nutrients can reach toxic levels in excessively low pH environments resulting in micronutrient toxicity.

Nutrient availability in high pH substrates (> 5.0 pH) poses the opposite problem. The bioavailability of essential nutrients is decreased, and plant uptake is restricted resulting in micronutrient deficiencies.

Most native plants tend to grow best at pH values ranging from 5.5 to 6.5 (Jacobs et al. 2008). Within the target pH range (5.5 to 6.5), the availability of micro and macro nutrients is maximized. Because of the direct influence pH and EC have on the growth potential of cultivated crops in the nursery, the effect biochar amendments have on properties like pH and EC are of significant interest. Alterations to substrate characteristics will likely provide some explanatory support in observed plant growth responses to increasing proportions of biochar amendments. The importance of managing the pH of the growing substrate is essential, as responsibly managing plant nutrition is important in producing a quality, merchantable seedling, but also in terms of minimizing environmental impacts associated with excessive fertilizer runoff from production nurseries (Landis and Dumroese 2006).

The electrical conductivity (EC) of the growing substrate is a measure of the amount of electricity that a solution will conduct. In the nursery, the capacity of a water solution to conduct electricity enables nursery practitioners to effectively gauge the amount of fertilizer (soluble salts) present in a growing substrate. This is an effective practice because all fertilizers are taken up by plants as electrically charged ions. Therefore, the ability of a substrate solution to conduct electricity measures the amount of charged nutrients available for plant uptake (Landis and Dumroese 2006).

Container nursery practice relies on constant nutrient inputs but care must be taken to avoid excessive amounts of salts from accumulating and damaging plant roots. At extreme concentrations, potential exists to reverse the osmotic potential causing water to flow out of plant roots and into the growing medium. Subsequently, plants grown in substrates with excessively high EC suffer poor plant growth and exhibit signs of water stress. Low EC values indicate that an insufficient amount of supplemented nutrients is present for plant uptake. Plants grown in nutrient deficient mediums are unable to obtain adequate micro and macro nutrients necessary for normal growth and exhibit tell-tale symptoms of nutrient deficiency. The ideal range for EC values in soilless media using the 2:1 extraction method is from 0.3 to 1.5 mS cm⁻¹ (Landis and Dumroese 2006).

The Study Species

One of the study goals is to provide information of plant growth responses to biochar amendments in northern Rocky Mountain native plant species, thus the four study species are deerhorn clarkia (*Clarkia pulchella* Pursh.), common blanketflower (*Gaillardia aristata* Pursh.), ponderosa pine (*Pinus ponderosa* Doug.), and Idaho fescue (*Festuca idahoensis* Elmer). Little to no information is available regarding the effect biochar has on northern Rocky Mountain native plant species. Including multiple study species provides an opportunity to expand current biochar research and will aid future biochar research in container nursery native plant propagation. The study species were not selected at random, but are components of the canopy and understory vegetation in a ponderosa pine ecotype. Furthermore, the study species have established uses in ecosystem restoration practices as common components of native seed mixtures, and are included in outplantings to increase ecosystem diversity, improve pollinator habitat, increase

wildlife forage, provide ground cover for erosion control, and reestablish ecosystem function in disturbed sites.

Additionally, by including species that span various growth habits (long-lived woody tree, annual herbaceous forb, perennial herbaceous forb, and a perennial graminoid) any information garnered will enable researchers the ability to infer potential plant growth responses in comparable species with comparable growth habits.

Clarkia pulchella, commonly known as deerhorn clarkia, ragged robin or pinkfairies, is an annual flowering herbaceous forb in the Onagraceae. This species grows to 15-42 cm tall and is commonly found in the Pacific Northwest. Deerhorn clarkia inhabits moderately dry locations, often in areas where the soil has experienced disturbance. *Clarkia pulchella* is found throughout western North America from British Columbia to Oregon, east to South Dakota, in valleys, foothills, and lower mountain elevations (Hitchcock and Cronquist 1973). Flowering time is from June to October. This species is included in native seed mixtures for prairie restoration projects. Their blooms are attractive to bees and wildlife (Craighead et al. 1963).

Festuca idahoensis, commonly known as Idaho fescue or bluebunch fescue, is a native perennial cool season grass in the Poaceae. Idaho fescue culms are erect and range in height from 0.3 to 1.0 m. Idaho fescue is one of the most commonly distributed grasses in the western States, and occupies much diversified habitats with the distribution extending to California, Colorado, Idaho, Montana, Nevada, Oregon, Washington, South Dakota, Utah, and Wyoming (Zouhar 2000). Although, *Festuca idahoensis* can be found at elevational extremes, it is most prevalent from about 1524 to 2439 m. This species grows well on all exposures and in a variety of soil conditions (pH ranging from 5.6 to 8.4).

F. idahoensis is considered one of the best forage grass species in Montana, and may dominate extensive portions of the landscape once established. Because of this it provides substantial forage for domestic livestock and multiple wildlife species (Zouhar 2000). Idaho fescue is a late seral community dominant species, and as such, their inclusion in restoration reseeded is important to reestablish ecosystem function (Chambers 1987). Despite the competitive disadvantage many native species have during the restoration of disturbed sites, native species such as Idaho fescue are an important component of restoration seed mixtures to rehabilitate areas of disturbance. Other small scale restoration projects have used fescue propagules in the greenhouse to reestablish grassland sites (Antieau and Gaynor 1990, Youtie 1992). *F. idahoensis* is slow to establish, but once established vigorous growth of above and below ground biomass provide effective ground cover and yield copious amounts of tough, fibrous roots that control erosion and improve soil structure (Hafenrichter et al. 1968). Other advantages of the species include its capacity to retard or prevent the noxious weed invasion once it is firmly established (Borman et al. 1990, Hafenrichter et al. 1968).

Pinus ponderosa, commonly known as ponderosa pine, western yellow pine, and bull pine, is a large long-lived native forest tree in the Pinaceae. Trees reach maturity at ages ranging from 70 to 250 years, and range in height from 30 to 50 m tall. Diameter at breast height in mature trees ranges from 0.6 to 1.3 m. Ponderosa pine is one of the most common, widely-distributed pine species, ranging from southern British Columbia to New Mexico (Kershaw et. al 1998). Uses for this species are diverse, ranging from value as a major timber resource, to providing wildlife habitat, to recreational use, and for their esthetic value. It grows in a diversity of soil types and conditions (pH ranging from 5.0 to 9.0). This species thrives in hot, dry locations and once established is considered to have good drought tolerance. Ponderosa pine is a climax

species in lower elevation coniferous forests, and a mid-successional species in higher elevations where other competitive conifer species can grow (Juncus 1998).

Gaillardia aristata, commonly known as blanketflower and common gaillardia, is a native perennial, tap-rooted wildflower with showy, yellow ray flowers and reddish brown central disk flowers. Plants are pubescent and grow 20 to 70 cm tall (Kershaw et al. 1998). Blanketflower is found in grasslands, woodlands, and montane meadows on sunny, well-drained sites. The natural range of this species extends from Canada to Colorado, east to the Dakotas and west to the Cascade Mountains in Washington (Marlowe and Hufford 2007). It grows well on a variety of soil types, and tolerates a soil pH ranging from slightly acidic to mildly alkaline. A wide variety of pollinators and beneficial insects rely on *Gaillardia* as a source of pollen and nectar for food, as well as for resting and cover. This species is a component of many northern dry grassland ecosystems.

Gaillardia aristata is useful for rehabilitating disturbed sites by contributing species diversity to native seed mixtures and native plant outplanting for restoration (Winslow 2011). Ecosystems with a diversity of functionally diverse species benefit from increased resistance to noxious weed invasion (Maron and Marler 2007). For example, Callaway et al. (2004) found *Centaurea stoebe* ssp. *macranthos* biomass was lower when grown in competition with *Gaillardia aristata*. Furthermore, native, deep-rooted forb species such as *Gaillardia* capture soil moisture and nutrients making them less available for weed establishment (Pokorny 2005).

Summary

Climate change is one of the most pertinent challenges facing the modern world. A multitude of mitigation strategies have been suggested, but few have potential to mitigate climate change in the next half century. Recent interest in climate change mitigation, supported by the

Terra Preta phenomenon, has brought to light the potential for biochar to sequester carbon in soils while enhancing plant growth (Atkinson et al. 2010, Laird et al. 2010, Warnock et al. 2010).

Actively implementing climate change mitigation strategies, coupled with altering current practices to promote nursery sustainability, are necessary. As of late, the sustainability of many nursery practices has come into question, and new, more sustainable options for replacing traditional peat-based growing mediums are emerging. Biochar-based growing media are potential replacements for less sustainable peat-based media.

Potential exists to shift modern horticultural reliance on peat-based media by incorporating biochar, and research efforts as of late have expanded to include the investigation of biochar application to soilless growing media (Dumroese et al. 2011, Fonteno and Jackson 2011, Graber et al. 2010, Headlee et al. 2014, Kaudal et al. 2015, Northup et al. 2013, Steiner and Harttung 2014, Tian et al. 2012, Vaughn et al. 2013). Limited research and varied results has hindered a wide spread shift toward biochar from peat-based growing media in container nursery production. Existing research in soilless growing media largely focus on crop or horticultural species, but results show positive media and plant responses to biochar amendments. Studies of the growth responses of native plant species in biochar-based substrates are scarce, but important if the full potential of biochar application in the nursery is to be realized.

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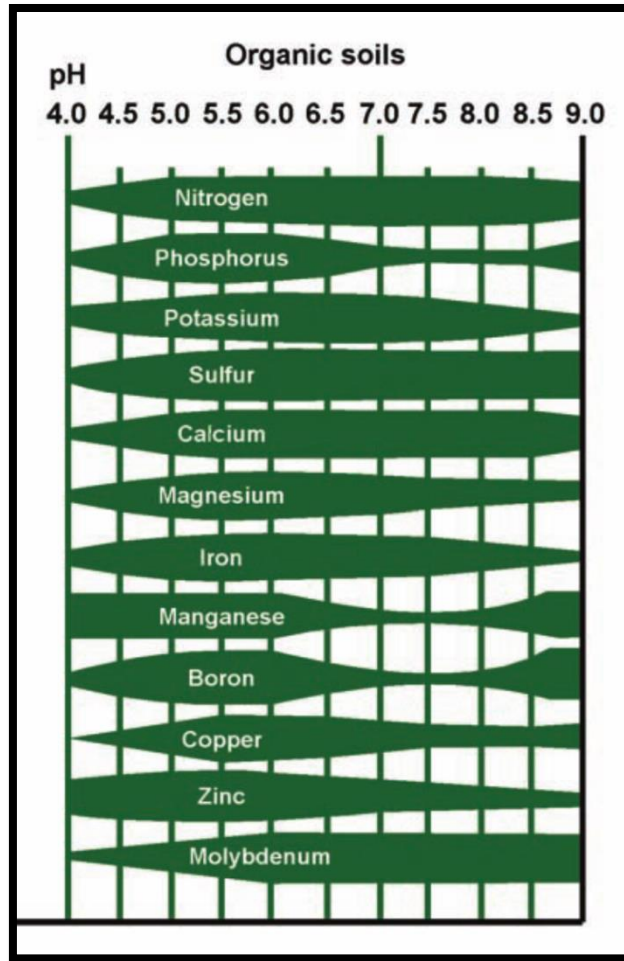


Figure 1. pH and macro and micro nutrient availability in organic soils. Nutrient availability is largely a function of media pH, and is maximized in the pH range of 5.5 to 6.5. Source: Jacobs et al. 2008).

CHAPTER 2: PLANT GROWTH, PH AND ELECTRICAL CONDUCTIVITY RESPONSES TO BIOCHAR AMENDMENTS TO SOILLESS MEDIA IN FOUR NORTHERN ROCKY MOUNTAIN NATIVE PLANT SPECIES WITH DIFFERENT LIFE FORMS

Abstract

A study was conducted to better inform nursery practitioners of the potential benefits of biochar application in common nursery media for native plant propagation. Biochar – a carbon-rich, recalcitrant charred organic co-product of the bioenergy pyrolysis process – has emerged as a promising potential replacement for various components of soilless media, namely peat, perlite, and vermiculite, in nursery seedling propagation. A strong conceptual basis exists for biochar as a nursery media amendment, but empirical data on biochar-based plant propagation is limited. This greenhouse study examined the effects of biochar displacement of standard soilless nursery media at rates of 0%, 15%, 30%, and 45% (percent volume composition) on propagation of four northern Rocky Mountain native plant species: deerhorn clarkia (*Clarkia pulchella* Pursh.), common blanketflower (*Gaillardia aristata* Pursh.), ponderosa pine (*Pinus ponderosa* Doug.), and Idaho fescue (*Festuca idahoensis* Elmer). Biochar at any level generally resulted in few differences in plant growth or media chemistry. Seedling biomass production with biochar treatment was either equivalent to the standard media (control), or in the case of *Festuca*, was slightly less. All plant growth parameters for *Festuca*, except longest leaf length, resulted in significantly lower values for all the biochar treatment levels. Final seedling height in the *Pinus* 30% treatment group had significantly taller seedlings, but no effect on mean total biomass. For all the species, media chemistry (pH and EC) showed little variation, and no clear trends resulting from biochar treatments emerged. Significant differences were found indicating the *Pinus* and *Gaillardia* un-amended media had higher mean pH than the other biochar treatments, but the overall range of pH values was small, and did not result in any apparent negative effects on plant growth. Although few benefits of biochar incorporation were identified, this research shows that biochar can reduce watering frequency and suitably displace up to 45% standard peat, perlite, and vermiculite media without any decrease in plant biomass growth for three of the study's four species.

Introduction

Native plant revegetation has quickly become an important component of ecosystem restoration projects in the northern Rocky Mountains, requiring an increasing supply of nursery seedlings to meet a growing demand. Concurrently, a focus on the sustainability of native plant propagation practices has grown, including a global movement to reduce the amount of unsustainable growing media components such as peat, perlite and vermiculite used by container nursery practitioners. While the desire to reduce the use of peat started as movement to responsibly manage peat harvesting, efforts to mitigate climate change have emphasized the role of intact peat ecosystems as important global carbon (C) sinks.

One material showing promise as a replacement for peat (Caron and Rochefort 2013, Tian et al. 2012, Vaughn et al. 2013, Steiner and Harttung 2014), perlite (Northup 2013) and vermiculite (Dumroese et al. 2011, Headlee et al. 2014) is biochar (Altland 2014). Biochar is a co-product of the bioenergy pyrolysis process during which biomass is heated in low-oxygen to zero-oxygen environments resulting in carbon-rich, recalcitrant charred organic matter. All biochar is not created equally; its characteristics are dependent on pyrolysis conditions and feedstock sources. In native plant nursery propagation, biochar may be a potentially beneficial amendment for standard growing media, which may bring benefits to plant productivity, reduce reliance on unsustainable media components, and incorporate biosequestration into restoration practices.

Biochar as a nursery media amendment is a relatively new application with many unknowns, but the conceptual basis for the application of biochar to field soils is supported by fire science and the existence of the Amazonian Anthrosols of South America (also known as Amazonian Dark Earths (ADEs) or *Terra Preta*; Lehmann 2007). Extensive research efforts on

the functioning of *Terra Preta* soils have identified biochar additions as the primary mechanism explaining the enhanced, sustained fertility and carrying capacities of these soils. They are the foundation of the resurgence of interest in charcoal application as a soil amendment and its potential economic and environmental value to modern society (Van Zwieten et al. 2010).

Biochar's potential to enhance nutrient dynamics in native plant propagation and incorporate climate change mitigation through carbon biosequestration into the restoration outplanting process is a much needed area of research. Past studies show a range of plant growth responses (-29% to +324%) resulting from a wide range of biochar application rates (Glaser et al. 2002) produced from a multitude of feedstock types. Effects of biochar on plant growth are influenced by multiple non-independent factors, including biochar rate, plant species, and soil and/or media characteristics. As a result, species-, soil- and media-specific studies are needed to examine how biochar-amended media may best benefit native plant propagation. Yet, information about biochar-based plant propagation is limited. The focus of current biochar studies is on plant growth responses of agricultural and horticultural species. Studies including plant growth responses of native plant species are limited to biochar application to field soils.

We conducted a greenhouse study to examine the effects of biochar on the growth of four northern Rocky Mountain native plant species: deerhorn clarkia (*Clarkia pulchella* Pursh.), common blanketflower (*Gaillardia aristata* Pursh.), ponderosa pine (*Pinus ponderosa* Doug.), and Idaho fescue (*Festuca idahoensis* Elmer). We investigated effects on plant growth and media properties by assessing whether biochar amendments could; (1) improve standard nursery media properties leading to enhanced seedling productivity, and (2) replace or amend other commonly used nursery media products (such as peat) in the propagation of northern Rocky Mountain native plants. Our objective was to assess the potential utility of biochar to native plant

propagation. To meet this objective we established a 4 x 4 x 4 multifactorial (species x treatment x replicate) experimental design to address the following questions:

1. What are the potential effects of biochar on the growth of four Rocky Mountain native plant species 1) *Pinus ponderosa*. 2) *Gaillardia aristata* 3) *Clarkia pulchella* , and 4) *Festuca idahoensis*.
2. How are pH and electrical conductivity media properties of the nursery media affected by varying levels of biochar amendment?

Materials & Methods

Study Design and Treatments

The experiment was conducted during the course of one growing season, from May to December, in 2012 in the College of Forestry and Conservation Memorial Greenhouse (Lat. 46.85863 Long. -113.98391) at the University of Montana (Missoula, MT). Greenhouse daytime temperatures were maintained between 21 and 25 °C until the end of the active growth phase, when temperatures were reduced to encourage hardening off.

The control medium used in our study is commonly used in nurseries and is comprised of a 3:1:1 ratio (by volume) of peat, perlite, and vermiculite. The container (cell) used was a Ray Leach Supercell™ with a diameter of 3.8 cm, depth of 21 cm and a volume of 164 ml. A total of 98 cells can fit within a single tray. We selected this particular nursery container type because it is common in production nurseries, easily handled and the cells can be rearranged.

Treatments utilized CQuest biochar (Image 1) produced by Dynamotive Energy Systems Corporation (Richmond, BC, Canada). The 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). Biochar total carbon, nitrogen and C:N, total element concentration

(mg kg⁻¹) characterization, and mean particle size distribution (%) data are provided in Tables 1-3.

The following biochar treatments were prepared on a percent total volume basis for each of the four study species, and replicated four times. The biochar was fully incorporated into the growth media for each treatment at rates of 0%, 15%, 30% and 45% volume. To minimize inconsistencies during the media preparation process, four replicate media batches were mixed that provided enough media to fill one complete replicate for all of the species. The cells for each replicate were filled in thirds and packed to a uniform density.

Plant Materials and Propagation

Seeds were acquired from multiple sources, and care was taken to identify local seed sources. *Festuca* seeds were acquired from Westland Seed: Farm and Garden Ranch Center (Ronan, MT). *Gaillardia* and *Clarkia* seeds were obtained from Native Ideals Seed Company (Arlee, MT). *Pinus* seeds were obtained from the Inland Empire Tree Improvement Cooperative seed orchard (Missoula, MT). Only the *Pinus* required stratification prior to sowing. *Pinus* seeds were surface sterilized in an 8:1 (v:v) bleach soak for 8 minutes, and placed in a running water soak for 48 hours prior to stratification (3°C) for 45 days in an incubator. Seeds of all species were sown directly into prepared treatment containers in late April and early May. To ensure enough complete experimental units, multiple seeds were sown in each cell. Germinants were thinned as necessary to establish one seedling per cell. Additionally, 25 extra cells of each species-treatment combinations, as well as two extra “filler” replicates were sown to minimize the need for transplanting, and ensure the successful establishment of seedlings in four complete replicates.

The block weight method was used to govern irrigation and fertigation events. Block weights are commonly used in production nurseries to establish appropriate times to irrigate/fertigate (for details, see Landis and Wilkinson 2009). Container mass for filled, sown cells were determined, and the entire block was weighed before and after each irrigation/fertigation event. Anticipating differential dry-down periods among treatments, we designated 75% of the container mass for each unit as a standard point at which each unit was irrigated/fertigated. After each destructive sampling date and weekly fertigation event, block field capacity weights were readjusted.

Fertilization consisted of General Hydroponics' Flora Duo two-part nutrient system (Part A: 5-0-6; Part B: 1-5-4) in a 1:1 ratio, once weekly. Fertilizer concentrations were determined to achieve an application rate of 150 ppm of nitrogen (N) during each fertigation event. Once the *P. ponderosa* set bud in early September, we decreased the ppm-N applied to 75 ppm for the *Pinus* and *Gaillardia*.

Data Collection and Analysis

A multi-factorial block design was implemented, with factors corresponding to the biochar treatment, species, and harvest date, and with blocks corresponding to each replicate. The variables measured throughout the study included: growth measurements, final media analyses of pH and EC, and plant tissue nutrient concentrations.

Each replicate was harvested on four separate dates, and each harvest date combined three composite seedlings (Figure 2). For example, replicate 1 cells for the *Pinus* control treatment were contained in a single tray. Four species with four treatments and four harvests with three composite seedlings each ($4 \times 4 \times 4 = 64$ trays) produced 64 trays, 16 trays for each species. Each study tray had a total of 12 seedlings (3 composite seedlings for each of four

harvest dates) for each species/treatment combination (64 trays x 12 seedlings/tray = n = 768 total seedlings).

Plant growth parameters and destructive sampling dates for each species are summarized in Table 4. Variation in growth forms among the study species led us to quantify plant growth responses using species-specific metrics. For example, height measurements over time were appropriate for species that exhibit marked vertical growth such as the *Pinus* and *Clarkia*; however, for species in which height is a poor overall indicator of growth, as is the case with *Festuca* and *Gaillardia*, alternative growth measures were implemented.

A total of four destructive harvests for each plant group (short-season species vs. long-season species) were undertaken at either 3, 6, 9 and 12 weeks (*Clarkia* and *Festuca*) or 8, 14, 20 and 26 weeks (*Pinus* and *Gaillardia*). At each scheduled destructive harvest, three composite seedlings were processed and measures were averaged for each replicate. Roots and shoots were separated, rinsed with deionized water, and dried in a forced air drying oven at 70 °C for 48 hours. Dry weights were determined using an Ohaus analytical balance (Explorer EO1640, Pinebrook, New Jersey). Samples were then stored in plastic bags for tissue preparation for plant tissue nutrient analysis. Biomass was pre-processed using a standard coffee grinder, and finished by hand, if necessary, with a mortar and pestle. Ground tissue samples were sent to J.R. Peters Inc. Laboratory (Allentown, PA) for tissue analysis of % total N, %P, %K, Ca, Mg, Fe, Mn, B, Cu, Zn, Mo, Al and Na.

Our study focused on evaluating the effect different rates of biochar amendments have on media pH and EC. The 2:1 method was used to measure the EC and pH of the final harvest media samples (for details see 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

(model # 2265FS, Spectrum Technologies, Aurora, IL) and a pHep 4 temperature adjusted pH meter (model # HI 98127, Hanna Instruments, Woonsocket, RI), respectively. Both the pH and EC meters were recalibrated after the completion of each replicate, and rinsed thoroughly with deionized water to maintain quality control and prevent contamination between samples.

Statistical Approach

In general, we expected that the incorporation of biochar would enhance media chemical properties, plant growth, and plant tissue nutrients compared with the standard biochar-free media mix. The statistical software R (version 3.1.0, Boston, MA) was used to test of the following hypotheses:

1. Native plant species grown in biochar-amended nursery media will produce significantly greater biomass production (mass; grams) than those grown in the control treatments;
2. Rocky Mountain native plant species grown in biochar will exhibit plant tissue nutrient concentrations for % total N, %P and %K that are greater than plants grown in the control treatments;
3. Biochar amendments will alter key nursery media properties: pH and electrical conductivity (EC).

Wherever possible, we applied analysis of variance (ANOVA) F-tests to identify significant differences ($\alpha=0.05$) among treatments for all response variables, separately for each of the species, using transformed and untransformed data as appropriate. We used ANOVA to analyze the final harvest data for all response variables because the sample sizes were equal, and ANOVA F-tests are robust against variance heteroscedasticity when sample sizes are equal. Tests were performed on final harvest data. The normality assumption was evaluated via Shapiro-Wilk normality test (Shapiro and Wilk 1965) at $\alpha=0.05$. The assumption of equality of

variances was evaluated via Bartlett's test for homogeneity of variances (Snedecor and Cochran 1983) at $\alpha=0.05$. For those response variables that did not meet ANOVA model assumptions, particularly the normality assumption, Kruskal-Wallis (1952) non-parametric tests of stochastic dominance were utilized to identify significant differences among treatments.

Details for each species are as follows:

For *Clarkia*, untransformed mean root biomass, final height, media pH, % total N, met both ANOVA assumptions. Log transformations to plant tissue %P and %K normalized the distributions, and subsequently met both assumptions. However, no transformations successfully resolved the issue of variance heteroscedasticity present in the mean shoot biomass, mean total biomass, and R:S response variables. No data transformation resolved either the normality or the unequal variances for the EC measures, so non-parametric Kruskal-Wallis tests were used to identify significant differences ($\alpha=0.05$).

For *Festuca*, untransformed data for mean shoot biomass, seedling height, R:S, media pH, media EC, plant tissue % total N, %P, and %K all met both ANOVA assumptions. Log transformations to the mean root biomass, mean total biomass, and R:S data successfully resolved deviations from normality. All of the response variable data, untransformed and log-transformed, met the equal variances assumption.

For *Gaillardia*, untransformed data for mean shoot biomass, mean root biomass, mean total biomass, leaf count, R:S, media pH, and plant tissue % total N, %P, %K all met the normality and equal variance model assumptions. Log-transformation to the media pH variable successfully resolved deviations from normality, and met the equal variance assumption.

For *Pinus* data, untransformed data for mean shoot biomass, seedling height, R:S, and plant tissue % total N, %P and %K all met both model assumptions of normality and equal

variances. No data transformations successfully resolved variance heterodescasticity in the mean root biomass and mean total biomass response variables. No data transformations successfully resolved deviations from normality observed in the media pH and media EC response variables, so Kruskal-Wallis non-parametric tests were employed.

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

Results

Growth Trajectories

The growth data gathered at each destructive sampling harvest was used to create biomass accumulation curves used to visually assess growth responses to biochar treatments for each species over time; no statistical analysis was done on biomass accumulation as a function of biochar treatment over time.

For *Clarkia*, the growth trajectories for each of the biochar treatments did not drastically differ from that of the control, particularly by the second harvest (Figure 3). The control group did have higher overall total biomass initially, but by the final harvest no treatment effect was apparent. By week 12, the 30% biochar treatment group had the greatest overall biomass accumulation ($\bar{x} = 7.35$ g) followed by the control group ($\bar{x} = 7.05$ g), 15% biochar group ($\bar{x} = 6.94$ g) and 45% group ($\bar{x} = 6.22$ g). One trend of interest is the apparent reduced biomass accumulation that occurred during early seedling establishment in seedlings grown in biochar amended standard soilless media. At the first harvest, all of the biochar treatment groups

exhibited markedly less biomass accumulation when compared to the control group. However, by the final harvest, mean total biomass for all of the treatments was comparable.

For *Festuca*, the growth trajectories for all treatments were similar (Figure 4). Unlike the *Clarkia*, *Festuca* biomass accumulation in biochar amended growing media did not differ from the control group. For the first two harvest dates, all groups mean total biomass accumulation was comparable. For the last two harvest dates, mean total biomass accumulation began to differentiate for each of the treatments. In the end, the control group had the greatest mean total biomass ($\bar{x} = 6.40$ g) followed by the 15% ($\bar{x} = 1.89$ g), 30% ($\bar{x} = 1.88$ g) and 45% ($\bar{x} = 1.64$ g) biochar treatment groups. The rate of biomass accumulation was similar for the control and 45% treatment groups, and started to increase over time. Contrasting this trend, the 15% and 30% biochar treatment groups exhibited increases in mean total biomass, but over time the rate of biomass accumulation began to slow compared to the other treatment groups.

For *Gaillardia*, the growth trends exhibited the greatest amount of variation by treatment when compared to the other study species (Figure 5). This was the only species that lost biomass for two of the intervals during the study. The first interval during which mean total biomass decreased from 5.65 g at week 14 to 5.15 g at week 20. All other treatment groups continued to accumulate biomass during this interval. The second observed decrease in mean total biomass was found in the control at week 26. Following both instances of mean total biomass decrease, biomass increased in the subsequent harvest. By the final harvest, the 30% biochar group ($\bar{x} = 7.13$ g) had the greatest mean total biomass followed by the 45% ($\bar{x} = 6.91$ g), 15% ($\bar{x} = 6.19$ g) and control groups ($\bar{x} = 4.67$ g) in decreasing order. Overall, the observed rate of biomass accumulation was most consistent in the 30% biochar treatment group. All of the other treatment groups' biomass accumulation rates slowed over time.

Compared to all of the other study species biomass accumulation curves, *Pinus* exhibited the least amount of variation in overall mean total biomass accumulation over time (Figure 6). The greatest mean total biomass was observed in the 30% biochar group at 5.14 g, followed by the 15%, 45%, and control groups having 4.14 g, 4.04 g and 4.01 g, respectively. Growth trajectories for the control group and 45% biochar treatment groups were almost identical, with the latter group having slightly greater final mean total biomass, 4.01 g and 4.04 g, respectively. Mean total biomass at week 26 was comparable for all treatment groups.

Watering frequency

No statistical analysis was conducted on watering frequency as a function of biochar treatment, but irrigation records indicate a common trend across species. For all species, biochar amendments to soilless media resulted in reduced watering frequency (compared with the controls) that was positively related to biochar level (Table 5). Comparing the controls with the 45% biochar group for all species combined, the controls overall required 39% more frequent irrigation to maintain field capacity mass. The greatest difference was observed for *Festuca*, where the control required nearly 55% more frequent irrigation than the treatment with the highest amount of biochar.

Final Harvest Tests

Clarkia pulchella

We found no significant effect of biochar treatment for *Clarkia* total biomass (Figure 7), nor any of the variables analyzed. Summary tables of response variable means and plant tissue nutrients are provided in Tables 6 and 7 (respectively).

Festuca idahoensis

For *Festuca*, biochar significantly affected total biomass (p-value = 0.008), root biomass (p-value = 0.003), bunch diameter (p-value = 0.047), and R:S (p-value = 0.014). The control group had significantly more total biomass (\bar{x} = 6.40 g) than the 15% (\bar{x} = 4.46 g, p-value = 0.016) and 30% (\bar{x} = 4.36 g, p-value=0.010) biochar treatment groups (Figure 8). The control treatment group also tended to have more total biomass than the 45% biochar treatment group (\bar{x} = 4.84 g). Results were similar for root biomass; the control group (\bar{x} = 4.10 g) yielded significantly more root biomass than the 15% (\bar{x} = 2.62 g, p-value = 0.017), 30% (\bar{x} = 2.30 g, p-value = 0.003), and 45% (\bar{x} = 2.61 g, p-value = 0.016) biochar treatment groups (Figure 9). Further, we found the control treatment group produced grasses with wider bunch diameters (\bar{x} = 31.93 mm) than the 15% treatment group (\bar{x} = 29.41 mm, p-value = 0.040) (Figure 10). The control treatment group also tended to produce seedlings with wider bunch diameters than the 30% (\bar{x} = 30.18 mm) and 45% (\bar{x} = 29.95 mm) biochar treatment groups. For R:S, control seedlings (\bar{x} = 1.80) had higher R:S than the 30% (\bar{x} = 1.12, p-value = 0.017) and 15% (\bar{x} = 1.20, p-value = 0.033) biochar treatment groups (Figure 11). The control treatment groups also tended to produce grasses with greater R:S than the 15% biochar treatment group (\bar{x} = 1.45). Summary tables of response variable and plant tissue nutrient means are provided in Tables 8 and 9 (respectively).

Gaillardia aristata

Media pH with *Gaillardia* was significantly (p-value = 0.003) affected by biochar amendment. The control treatment group media had significantly higher media pH (\bar{x} = 7.79) than the 30% (\bar{x} = 7.49, p-value=0.007) and 45% (\bar{x} = 7.49, p-value=0.006) biochar treatment groups (Figure 12), but was not different than the 15% biochar treatment group (\bar{x} = 7.65).

Although not significant, seedlings grown in the 30% biochar amended media tended to have more total biomass ($\bar{x} = 7.13$ g) followed by the 45% ($\bar{x} = 6.91$ g), 15% ($\bar{x} = 6.20$ g) and control ($\bar{x} = 4.67$ g) treatment groups (Figure 13). Summary tables of response variable and plant tissue nutrient means are provided in Tables 10 and 11 (respectively).

Pinus ponderosa

For *Pinus*, biochar significantly affected seedling height (p-value = 0.007), and plant tissue %K (p-value = 0.013) and media pH (p-value = 0.026). The 30% biochar treatment group ($\bar{x} = 19.76$ cm) had taller seedlings than all of the other treatment groups (Figure 14): Control ($\bar{x} = 16.55$ cm, p-value = 0.019), 15% ($\bar{x} = 16.12$ cm, p-value = 0.008) and 45% ($\bar{x} = 16.86$ cm, p-value = 0.035). For plant tissue %K, the 45% biochar treatment group plant tissues had higher tissue %K ($\bar{x} = 1.47\%K$) than the 15% biochar treatment group ($\bar{x} = 1.19\%K$, p-value = 0.033) (Figure 15). Plant tissue %K in the 45% biochar treatment group also tended to be higher than the 30% ($\bar{x} = 1.45\%K$) and control ($\bar{x} = 1.23\%K$) groups. The control group had significantly higher substrate pH ($\bar{x} = 7.42$) than the 45% biochar treatment group ($\bar{x} = 7.2$) (Figure 16). The pH of all of the study treatments was comparable and minimal variation existed. The control group media pH also tended to be slightly higher than the 15% ($\bar{x} = 7.24$), 30% ($\bar{x} = 7.21$) and 45% ($\bar{x} = 7.2$) biochar treatment groups. Biochar treatment level did not have any effect on final total biomass (Figure 17). Summary tables of response variable and plant tissue nutrient means are provided in Tables 12 and 13 (respectively).

Discussion

The body of biochar research in soilless substrates is far less comprehensive than studies conducted in mineral soils. Although biochar studies involving soilless media are limited, some studies do exist. Findings report positive correlations between biochar additions and plant growth

(Graber et al. 2010), resistance to pathogenic fungi (Zwart and Kim 2012) and nutrient retention (Altland and Locke 2012). The focus of many recent biochar studies examines the suitability of biochar to amend (Dumroese et al. 2011, Graber et al. 2010), and displace portions of soilless growing substrates by evaluating the effect on media properties and plant growth responses (Northup et al. 2013, Steiner and Harttung 2013, Vaughn et al. 2014). Studies such as these suggest the need for research aimed at investigating the role of biochar in the nursery, and suggest the biochar-based media may pose a realistic alternative for peat-based growing media.

Plant Growth

Our study results did not find any biochar treatment effect on plant growth (measured as total biomass), with the exception of *Festuca idahoensis*. In our study, growth parameters were neither enhanced nor diminished in *Clarkia*, *Gaillardia* and *Pinus*, which is similar to other findings with crop and horticultural species (Vaughn et al. 2013), but contrary to positive correlations in seedling dry weight reported by others (Graber et al. 2010, Headlee et al. 2014, Tian et al. 2012). Positive correlations in seedling biomass were not a result of enhanced seedling nutrition but from increased resistance to pathogenic fungi (Graber et al. 2010, Zwart and Kim 2012). Biochar can decrease nutrient leaching in soilless media (Altland and Locke 2012) but positive plant growth responses did not correlate with increased plant tissue nutrients (Graber et al. 2010) or electrical conductivity (Vaughn et al. 2013).

Differences in biochar properties, application rates and study species may explain contrasting results, and further indicates the need to standardize study parameters in future biochar research in soilless media (Mukome et al. 2013, Gundale and Deluca 2007). The properties of biochar vary according to feedstock and pyrolysis conditions (Downie et al. 2009, Novak et al. 2009, Rajkovich et al. 2011). Feedstock largely influences inherent nutrient

concentration and porosity, whereas pyrolysis conditions (temperature and heating time) influences C conversion to stable forms, pH, surface area and cation exchange capacity (Novak et al. 2009).

Although we observed many trends toward the biochar groups having overall higher total biomass compared to the controls, these differences were not significant. We noted substantial within treatment variation that may be potentially obscuring differences in total biomass resulting from biochar amendments. An extreme example this was observed in the *Gaillardia* 45% treatment group data set where mean total biomass was 6.91 g, but values ranges from 2.88 g to 10.79 g. Despite this, and because the biochar treatments were usually not statistically different from the control, we can conclude that biochar amendments do not negatively affect *Gaillardia* growth.

Media pH

The primary effect of pH on plant growth is its effect on nutrient availability. Most native plants tend to grow best at pH values ranging from 5.5 to 6.5 (Jacobs et al. 2008) because the availability of micro and macro nutrients essential for normal plant growth is maximized. Interestingly, 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 supplemental nutrients in excess (150 ppm-N). Studies indicate that biochar raises pH in soilless media (Steiner and Harttung 2014, Tian et al. 2012, Vaughn et al. 2013), allowing it to act as a suitable replacement for lime necessary to maintain adequate pH values in peat-based growing media (Northup et al. 2013, Steiner and Harttung 2014). In our study, however, biochar did not significantly raise pH at even the highest biochar treatment level compared to the control, and in a couple instances (*Gaillardia* and *Pinus*) the biochar treatment groups had lower pH (more acidic) values

compared to the control, albeit variation among treatments was small (7.79 to 7.49 in the *Gaillardia*; 7.42 to 7.2 in the *Pinus*). Possibly explaining the contrary trend in media pH could be more frequent irrigation in the control treatments and the high pH of the irrigation water used in the greenhouse (7.6) that caused the pH of the control medium to increase during the experiment to levels similar to those observed in biochar-amended media.

Electrical conductivity (EC)

In the nursery, routinely testing substrate EC enables nursery practitioners to effectively gauge the amount of fertilizer (measured as solubilized electrically charged ions) present in a growing substrate. One significant drawback of using native plant species is that there is no species-specific reference of baseline values for optimal pH and EC measures.

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, suitable for seedlings (BC Ministry of Agriculture and Food 1999) and our observed values (0.28 to 0.38 mS cm⁻¹) are at the low end of that range and adequate for seedling growth (Fisher and Argo 2005, Camberato et al. 2001).

EC can increase (Vaughn et al. 2013) or decrease (Steiner and Harttung 2014) with biochar additions, or, as was the case with our study, reveal no clear trend (Northup et al. 2013). Northup et al. 2013 conducted a 16 week 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, physical properties and plant growth (species not specified). They found pH increased as biochar rate increased and particle size decreased but there was no clear trend in media EC as a function of biochar rate or particle size. We found a similar lack in EC trends from biochar treatments over time.

Similarities in biochar rates, biochar particle size, media components and study duration in this study offer a better opportunity for comparison compared to other biochar studies with shorter sampling times, duration, and substantially lower application rates.

Interestingly, studies reporting increases in media EC did not report increases in plant growth (Vaughn et al. 2013), and vice-versa (Steiner and Harttung 2014). Mixed responses in electrical conductivity led us to conjecture three possible EC trend scenarios: (1) media EC would increase as biochar increased resulting in enhanced plant growth and seedling nutrition, or (2) media EC would increase as biochar increased but result plant poor plant growth and seedling nutrition, or (3) EC would decrease as biochar increased resulting in in poor seedling growth and nutrition. Variable effects of biochar on EC indicate increases do not always imply greater nutrient availability for plant uptake that may be explained by biochar induced increases to surface area and nutrient adsorption. In our study, no EC trend was found nor did biochar affect plant growth (except for *Festuca*) or seedling nutrition.

Biochar charging

During early seedling establishment we noted a lag in biomass accumulation for *Clarkia pulchella* for all the biochar treatment groups compared with the control group (Figure 2). This likely resulted from the need for biochar to charge with sufficient nutrients prior to making them available for plant uptake. Interestingly, and somewhat unexpected, none of the other study species exhibited this lag in biomass accumulation during seedling establishment, at least as could be determined with our sampling timeline. It is possible that biochar charging, in fact, occurred in the other species, but that the study timeline aligned especially well with the *Clarkia* enabling us to capture the charging effect.

The need for fresh biochar to charge is well known within the context of biochar application to field soils. Fresh biochars have low CEC values that increase over time as the surface of the biochar is exposed to air and water (Cheng et al. 2008, Cheng et al. 2006, Liang et al. 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 would anticipate 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 found no clear trend as a function of biochar amendment (Northup et al. 2013, Housley 2011). The lack of an observed biochar charging phenomenon in these studies may be explained by the established sampling timelines, as may have been the case in our study. Perhaps biochar charging occurs over less time in soilless media compared to field soil because of optimal water and nutrient conditions in the nursery. Measurement times may need to be adjusted accordingly to further investigate biochar charging in soilless media.

Watering frequency

Our study revealed that biochar amendments resulted in less frequent irrigation despite no 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 et al. 2009). Biochar properties, such as its highly porous nature and high surface area, affect soil

texture, aggregation and total porosity that can alter soil water retention (Downie et al. 2009, Glaser et al. 2002).

Biochar amendments to soilless media alter media physical properties in the same way (Kaudal et al. 2015, Northup et al. 2013). Biochar additions to soilless media alter pore size and distribution which in turn affects water retention capacity. For instance, biochar has a much smaller particle size compared to perlite and vermiculite. Biochar has greater overall total porosity and range of pore sizes (Downie et al. 2009). Perlite and vermiculite are added to soilless media to improve drainage. They have a larger particle size compared to biochar, and possess larger macropores that are less capable of retaining water, particularly at low volumetric water contents. As such, differences in particle size may explain the decrease in irrigation frequency observed in the biochar treatments. This suggests that biochar could be used to reduce overall 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 (Landis et al. 2010).

Implications for restoration

From a restoration standpoint, the true test of success of a nursery seedling grown in biochar-amended soilless media is increased seedling establishment and survival after outplanting. The advantages of biochar amendments to suboptimal field soils are well documented; biochar enhances water holding capacity (Iswaran et al. 1980) and nutrient dynamics (Lehmann 2003 a,b) when applied to soils. Therefore, it is logical to infer 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 subjected to field soil conditions, nutrient and water limitations. Outplanting has the potential for biochar benefits to water and nutrient dynamics to be realized.

Plant survival and growth are greatly influenced by soil moisture and its effect on nutrient uptake and translocation (Helenius et al. 2002). Root systems of outplanted seedlings must be able to acquire sufficient water from the soil to meet shoot transpiration requirements (Landis et al. 2010). In newly planted seedlings, water stress from insufficient soil moisture can reduce growth and increase mortality (Landis et al. 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 is used to increase water retained in the seedling plug during the outplanting process. Lower seedling mortality five months after outplanting was found in one trial implementing this technique that 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 of the northern Rocky Mountains. The need for outplanting studies investigating the potential advantage of biochar amendments on seedling establishment and survival are necessary. If positive correlations are found, this would add value to seedlings propagated in biochar-based media and thereby strengthen the market for biochar production. In addition, benefits of biochar-grown seedlings may offset the cost of sustainability associated with displacing a portion of peat-based growing media with biochar.

A significant diversity of biochars and growing media has led to varying effects on plant growth and media properties. As a result, extrapolating biochar effects found in this study to other native plant species, and/or other growing media should be limited. Conservatively, our study results are specific to this particular biochar and native plant species; yet, some inference is

possible. 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 (i.e. annual forb, perennial forb, long-lived tree, perennial graminoid). We advise that plant growth and media response studies are needed prior to adopting biochar-based media for large-scale native plant production.

The cost of being green

Utilizing biochar sources that are readily available for purchase on the open market would be advantageous. Companies such as Interra Energy are currently in the research and design phase of producing more efficient and cost effective bulk biochar for purchase. An informal survey indicates cost estimates for biochar range between \$118.30 and \$169.00 per cubic yard. Using published cost estimates for peat, perlite and vermiculite (Greenhouse Product News; Boyle 2006), a typical 3:1:1 peat:perlite:vermiculite (v:v:v) media mix costs \$54.54 per cubic yard. At the low end of the cost range, biochar-amended media at 15%, 30% and 45% levels would cost \$64.10, \$73.67, and \$83.23 per cubic yard (respectively); at the range's high end, they would cost \$71.71, \$88.88 and \$106.05 per cubic yard (respectively). These values correlate to relative cost increases (compared to standard media) of 1.18-1.31 times (15% biochar level), 1.35-1.63 times (30% biochar level), and 1.53-1.94 times (45% biochar level). Higher seedling survival rates would justify the increased price per seedling grown in amended media, offsetting the costs of biochar additions. This is only one cost comparison; values are only meant to provide some reference indicating the relative cost of incorporating biochar into container nursery production.

Conclusions

Our study is the first to investigate the effects of biochar amendments on the growth of an annual and perennial forb, a grass and a long-lived woody tree species native to the northern Rocky Mountains in a nursery setting. With the exception of *Festuca*, biochar amendments to this particular peat-based growing media had little effect on plant growth or media pH and EC. Biochar at all treatment levels, for all the study species, provided pH and EC values that allowed for normal plant growth, despite media property values being outside optimal recommended ranges for native plant species. In all cases, biochar reduced the need for irrigation, and this reduction was positively associated with the percent biochar level. *Festuca* findings may be viewed as unfavorable, it is, however, important to acknowledge a significant diversity in biochars exists, with equally diverse chemical and physical properties and so a form more suitable for *Festuca* may exist. Comparing irrigation frequency, however, differences between *Festuca* controls and the biochar treatment may explain decreased plant growth. In general, neutral effects of biochar on plant growth, pH and EC indicates biochar-based growing media have chemical and physical properties suitable for container native plant propagation.

Overall, evidence garnered from this study supports the suitability of biochar to displace up to 45% of standard peat, perlite, and vermiculite growing media without compromising the quality of propagated seedlings. Given the diversity in plant growth responses and alterations to growing media properties, the need to conduct biochar application in the nursery must be approached in a biochar-, media- and species-specific manner. Biochar in container seedling production can realistically contribute to climate change mitigation efforts using a multifaceted approach which embraces sustainable practices in native plant propagation.

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Table 1. Total carbon, nitrogen and carbon to nitrogen ratio for CQuest biochar.

	Total %C	Total %N	C:N
Biochar	74	0.37	199

1

Table 2. Total mineral concentrations for CQuest biochar (mg kg⁻¹).

	Al	B	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Zn
Biochar	163.6	17.02	4694	<0.117	98.42	7.97	1108	4340	509.2	139.4	<1.2	81.5	10.75	178.6	3.35	117.4	15.72

2

3

Table 3. Mean particle size distribution (%) for CQuest biochar determined by dry sieving.

	>5 mm	2-5 mm	1-2 mm	0.5-1 mm	<0.5 mm
Biochar	0	0.2	0.8	10.5	88.4

4

5

6 **Table 4.** Schedule of destructive sampling dates and growth parameters.

7 * Day 1 is defined as 2 weeks post sowing, and corresponds to first fertigation event.

<u>Species</u>	<u>Season length</u>	<u>Growth Habit</u>	<u>Growth parameters</u>	<u>Harvest Dates (from Day 1)*</u>
<i>Clarkia pulchella</i>	Short season	Annual herbaceous forb	Overall height (cm), shoot biomass, root biomass, final foliar nutrients.	3,6,9 and 12 weeks
<i>Festuca idahoensis</i>	Short season	Perennial graminoid	Length of longest leaf, bunch diameter, shoot biomass, root biomass, final foliar nutrients.	3,6,9 and 12 weeks
<i>Pinus ponderosa</i>	Long season	Perennial herbaceous forb	Overall height (cm), shoot biomass, root biomass, final foliar nutrients.	8,14,20 and 26 weeks
<i>Gaillardia aristata</i>	Long season	Long-lived woody tree	Number of true leaves, shoot biomass, root biomass, final foliar nutrients.	8,14,20 and 26 weeks

8

9 **Table 5.** Number of irrigations applied during the experiment to maintain treatments at 75%
10 field capacity based on total container mass.

Biochar treatment	<i>Clarkia</i>	<i>Festuca</i>	<i>Gaillardia</i>	<i>Pinus</i>
Control	38	31	53	49
15%	36	26	51	44
30%	32	21	44	39
45%	27	20	41	37

11

12 **Table 6.** Mean values for the *C. pulchella* response variables; std. error values are provided in the parentheses;* denotes p-values are
 13 for log data transformations, ** denotes p-values are for Kruskal-Wallis tests.

Response Variable Summary Table for <i>Clarkia pulchella</i>					
	Control (0%)	15%	30%	45%	p-value
Mean Total biomass (g)	7.05 (0.47)	6.94 (0.45)	7.35 (0.49)	6.228 (0.05)	0.3
Mean Shoot biomass (g)	4.41 (0.43)	4.34 (0.28)	4.47 (0.17)	4.31 (0.05)	0.655
Mean Root biomass (g)	2.65 (0.19)	2.61 (0.17)	2.60 (0.38)	1.92 (0.09)	0.132
Mean Final height (cm)	38.28 (0.68)	36.28 (2.13)	35.97 (2.06)	36.13 (2.57)	0.827
R:S	0.62 (0.09)	0.60 (0.01)	0.54 (0.07)	0.45 (0.03)	0.198
pH	7.25 (0.15)	7.30 (0.09)	7.40 (0.05)	7.31 (0.05)	0.735
EC (mS cm⁻¹)	0.28 (0.07)	0.32 (0.08)	0.29 (0.06)	0.27 (0.01)	0.8261**

14

15 **Table 7.** Mean plant tissue nutrient concentrations by treatment for *C. pulchella*; std. error values are given in parentheses.

Mean Foliar nutrient concentrations for <i>C. pulchella</i>													
	% Total N	%P	%K	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Bo (ppm)	Zn (ppm)	Mo (ppm)	Al (ppm)	Na (ppm)
Control (0%)	1.63 (0.03)	0.54(0.05)	2.28 (0.13)	2.59 (0.06)	0.65 (0.04)	50.28 (0.46)	106.55 (4.30)	263.5 (4.37)	18.85 (0.75)	35.2 (1.09)	4.44 (0.53)	44.8 (2.76)	398.25 (4.14)
15%	1.63 (0.06)	0.43 (0.02)	2.18 (0.07)	2.23 (0.04)	0.51 (0.02)	49.98 (0.59)	81.63 (2.03)	144.23 (3.45)	15.98 (0.63)	27.38 (0.86)	3.61 (0.33)	44.9 (3.40)	375.5 (15.39)
30%	1.69 (0.03)	0.45 (0.02)	2.31 (0.09)	2.19 (0.07)	0.54 (0.02)	47.2 (0.51)	57.8 (0.70)	85.2 (1.91)	12.78 (0.37)	25.68 (0.79)	3.26 (0.35)	29.43 (0.46)	424.75 (10.56)
45%	1.66 (0.06)	0.53 (0.05)	2.28 (0.27)	2.71 (0.19)	0.60 (0.03)	53 (0.67)	64.18 (2.12)	118.08 (2.33)	14.63(0.82)	28.13 (0.71)	3.55 (0.26)	50.73 (9.72)	602 (5.47)

16

17 **Table 8.** Mean values for the *F. idahoensis* response variables; std. error values are provided in the parentheses;* denotes p-values are
 18 for log data transformations, ** denotes p-values are for Kruskal-Wallis tests.

Response Variable Summary Table for <i>Festuca idahoensis</i>					
	Control (0%)	15%	30%	45%	p-value
Mean Total biomass (g)	6.40 (0.66)	4.46 (0.14)	4.36 (0.27)	4.84 (0.27)	0.00829*
Mean Shoot biomass (g)	2.30 (0.34)	1.84 (0.16)	2.06 (0.17)	2.24 (0.22)	0.217
Mean Root biomass (g)	4.10 (0.52)	2.62 (0.17)	2.30 (0.16)	2.61 (0.18)	0.0029*
Mean Length of Longest leaf (cm)	18.18 (0.28)	16.35 (0.42)	18.20 (0.31)	17.85 (0.40)	0.311
Mean Bunch Diameter (mm)	31.93 (0.42)	29.41 (0.32)	30.18 (0.40)	29.95 (0.95)	0.0469
R:S	1.80 (0.18)	1.45 (0.16)	1.12 (0.05)	1.18 (0.10)	0.0143*
pH	7.25 (0.08)	7.25 (0.04)	7.25 (0.05)	7.43 (0.01)	0.205
EC (mS cm⁻¹)	0.38 (0.20)	0.34 (0.04)	0.35 (0.04)	0.28 (0.38)	0.946

19

20 **Table 9.** Mean plant tissue nutrient concentrations by treatment for *F. idahoensis*; std. error values are given in parentheses.

Mean Foliar nutrient concentrations for <i>F. idahoensis</i>													
	% Total N	%P	%K	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Bo (ppm)	Zn (ppm)	Mo (ppm)	Al (ppm)	Na (ppm)
Control (0%)	1.97 (0.06)	0.31 (0.06)	1.98 (0.08)	0.48 (0.09)	0.18 (0.02)	15.85 (0.39)	56.13 (1.49)	219.75 (3.00)	13.12 (2.03)	31.33 (0.76)	2.54 (0.43)	40.3 (2.30)	165 (3.56)
15%	2.10 (0.12)	0.34 (0.04)	2.04 (0.22)	0.49 (0.09)	0.20 (0.01)	23.1 (0.61)	109.3 (3.83)	184.25 (3.53)	9.31 (0.70)	27.75 (0.59)	3.37 (1.37)	176.18 (39.45)	128.63 (3.06)
30%	2.12 (0.06)	0.37 (0.07)	2.18 (0.04)	0.45 (0.05)	0.18 (0.03)	22.98 (0.31)	126.83 (5.23)	134.5 (2.75)	10.66 (0.27)	26.1 (0.77)	4 (0.33)	138.08 (7.78)	145.35 (4.21)
45%	2.09 (0.08)	0.33 (0.06)	2.09 (0.13)	0.50 (0.05)	0.18 (0.01)	25.7 (0.20)	158.03 (3.21)	193.75 (1.99)	9.92 (0.56)	27.8 (2.62)	2.36 (0.24)	207.23 (7.95)	135 (2.23)

21

22 **Table 10.** Mean values for the *G. aristata* response variables; std. error values are provided in the parentheses;* denotes p-values are
 23 for log data transformations, ** denotes p-values are for Kruskal-Wallis tests.

Response Variable Summary Table for <i>Gaillardia aristata</i>					
	Control (0%)	15%	30%	45%	p-value
Mean Total biomass (g)	4.67 (0.71)	6.20 (0.62)	7.13 (1.26)	3.91 (1.62)	0.438
Mean Shoot biomass (g)	1.16 (0.33)	1.59 (0.09)	1.57 (0.54)	1.44 (0.46)	0.523
Mean Root biomass (g)	3.51 (0.52)	4.61 (0.47)	5.56 (0.95)	5.47 (1.17)	0.401
Mean Final leaf count	37.58 (1.24)	39.58 (2.27)	58.58 (1.81)	42.17 (1.83)	0.142
R:S	3.10 (0.28)	2.87 (0.28)	3.55 (0.23)	3.68 (0.37)	0.176
pH	7.79 (0.02)	7.65 (0.05)	7.49 (0.06)	7.49 (0.06)	0.0033
EC (mS cm⁻¹)	0.28 (0.14)	0.32 (0.23)	0.37 (0.20)	0.35 (0.37)	0.863*

25 **Table 11.** Mean plant tissue nutrient concentrations by treatment for *G. aristata*; std. error values are given in parentheses.

Mean Foliar nutrient concentrations													
	% Total N	%P	%K	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Bo (ppm)	Zn (ppm)	Mo (ppm)	Al (ppm)	Na (ppm)
Control (0%)	1.55 (0.06)	0.29 (0.13)	2.14 (0.15)	2.52 (0.15)	0.80 (0.08)	152.75 (1.64)	54.7 (1.08)	146 (1.69)	11.54 (0.68)	19.7 (0.88)	6.78 (0.63)	10.23 (1.33)	177.25 (1.54)
15%	1.46 (0.15)	0.24 (0.13)	1.98 (0.09)	2.64 (0.09)	0.81 (0.09)	125 (0.61)	49.38 (0.80)	101.9 (3.05)	12.25 (0.38)	16.9 (0.61)	6.83 (0.45)	11.18 (2.49)	199.25 (0.79)
30%	1.64 (0.16)	0.32 (0.16)	2.04 (0.16)	2.55 (0.11)	0.71 (0.14)	122.5 (3.11)	79.55 (6.64)	65.58 (1.73)	13.22 (0.65)	19 (1.57)	5.54 (0.38)	41.20 (16.20)	186 (1.05)
45%	1.63 (0.21)	0.26 (0.05)	1.95 (0.20)	2.63 (0.10)	0.76 (0.18)	138.5 (0.41)	54.8 (0.54)	102.03 (1.28)	11.73 (0.34)	20.35 (1.65)	5.38 (0.52)	8.43 (0.82)	195.25 (2.04)

27 **Table 12.** Mean values for *P. ponderosa* response variables; std. error values are provided in the parentheses;* denotes p-values are
 28 for log data transformations, ** denotes p-values are for Kruskal-Wallis tests.

Response Variable Summary Table for <i>Pinus ponderosa</i>					
	Control (0%)	15%	30%	45%	p-value
Mean Total biomass (g)	4.01 (0.51)	1.15 (0.37)	2.14 (0.51)	4.04 (0.06)	0.209
Mean Shoot biomass (g)	1.83 (0.39)	1.97 (0.25)	2.57 (0.38)	1.80 (0.09)	0.161
Mean Root biomass (g)	2.18 (0.31)	2.18 (0.24)	2.57 (0.19)	2.24 (0.03)	0.377
Mean Final height (cm)	16.55 (0.54)	16.12 (0.60)	19.76 (0.92)	16.88 (0.40)	0.00696
R:S	1.23 (0.21)	1.11 (0.15)	1.03 (0.17)	1.24 (0.07)	0.236
pH	7.42 (0.02)	7.24 (0.02)	7.21 (0.01)	7.2 (0.01)	0.02616**
EC (mS cm⁻¹)	0.31 (0.13)	0.29 (0.14)	0.27 (0.06)	0.28 (0.21)	0.4851**

29
 30 **Table 13.** Mean plant tissue nutrient concentrations by treatment for *P. ponderosa*; std. error values are given in parentheses.

Mean Foliar nutrient concentrations													
	% Total N	%P	%K	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Bo (ppm)	Zn (ppm)	Mo (ppm)	Al (ppm)	Na (ppm)
Control (0%)	2.50 (0.08)	0.34 (0.02)	1.23 (0.05)	0.55 (0.04)	0.16 (0.01)	42.6 (0.69)	40.93 (0.58)	179.75 (1.80)	6.64 (0.80)	34.8 (0.68)	5.23 (0.20)	43.58 (2.48)	68.73 (1.74)
15%	2.75 (0.12)	0.38 (0.05)	1.19 (0.06)	0.73 (0.08)	0.22 (0.02)	57.78 (1.40)	44.93 (0.57)	121.43 (1.72)	7.36 (0.46)	36.53 (1.69)	5.53 (0.58)	14.38 (1.06)	77.53 (2.95)
30%	2.41 (0.16)	0.38 (0.03)	1.45 (0.08)	0.75 (0.03)	0.22 (0.002)	54.48 (1.90)	39 (0.47)	91.28 (2.12)	6.17 (0.15)	30.68 (0.83)	5.56 (0.46)	8.11 (0.40)	66.5 (0.64)
45%	2.62 (0.07)	0.38 (0.02)	1.47 (0.05)	0.85 (0.03)	0.25 (0.01)	64.85 (0.49)	36.2 (0.30)	166.75 (2.55)	5.25 (0.05)	28.6 (0.76)	5.10 (0.68)	6.93 (0.18)	67.73 (3.04)

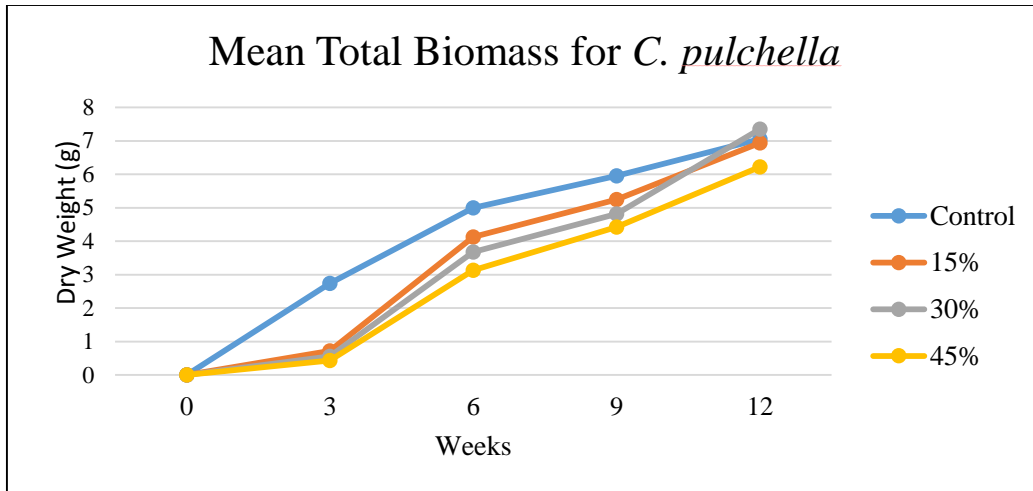
31

R1-4-a	R1-4-b	R1-4-c
PIPO	PIPO	PIPO
R1-3-a	R1-3-b	R1-3-c
PIPO	PIPO	PIPO
R1-2-a	R1-2-b	R1-2-c
PIPO	PIPO	PIPO
R1- 1-a	R1-1-b	R1-1-c
PIPO	PIPO	PIPO

33

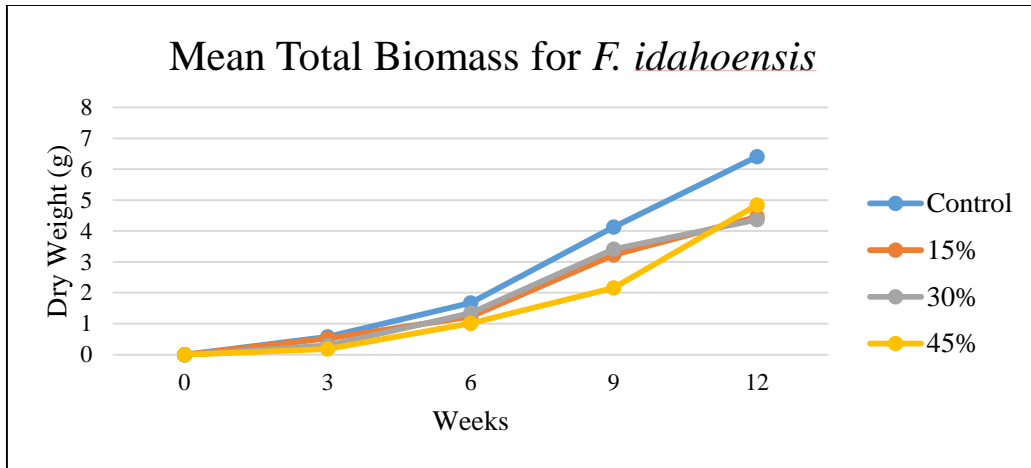
34 **Figure 2.** Illustration of the layout of experimental treatments applied in this study. In this
 35 example, *Pinus ponderosa* (PIPO) is the plant species. Treatment layout for all species was
 36 identical to the one below.

37 Note: labels represent Replicate (R1, R2, R3 or R4) - Harvest (1, 2, 3, or 4) - composite seedling
 38 (a, b, or c) and species abbreviation (e.g., PIPO for *Pinus ponderosa*). Treatments were
 39 distinguished by color coding the labels accordingly.



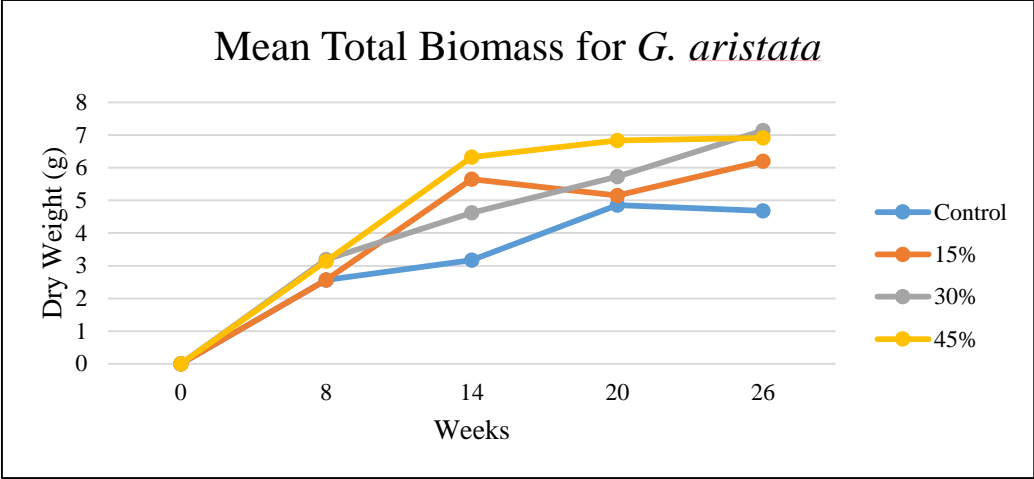
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41 **Figure 3.** Mean total biomass accumulation as a function of biochar treatment over time for *C.*
42 *pulchella*.



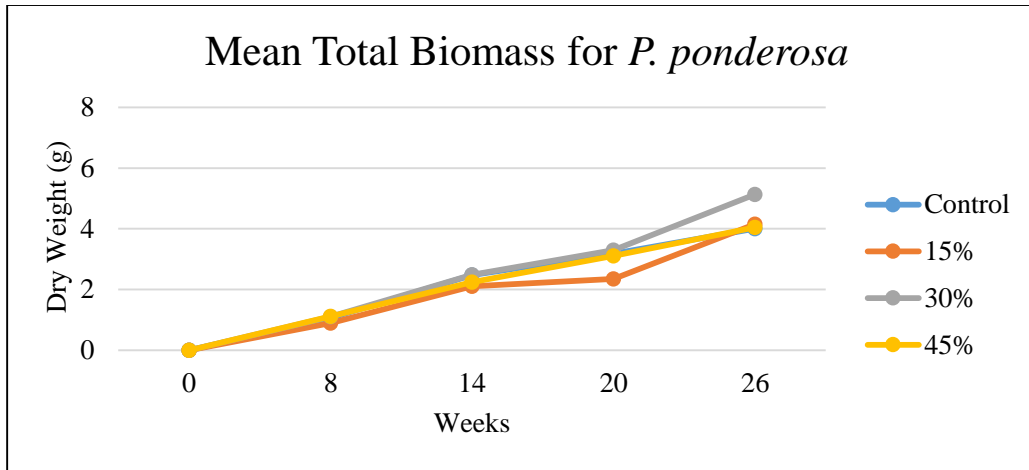
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44 **Figure 4.** Mean total biomass accumulation as a function of biochar treatment over time for *F.*
45 *idahoensis*.



46

47 **Figure 5.** Mean total biomass accumulation as a function of biochar treatment over time for *G.*
48 *aristata*.

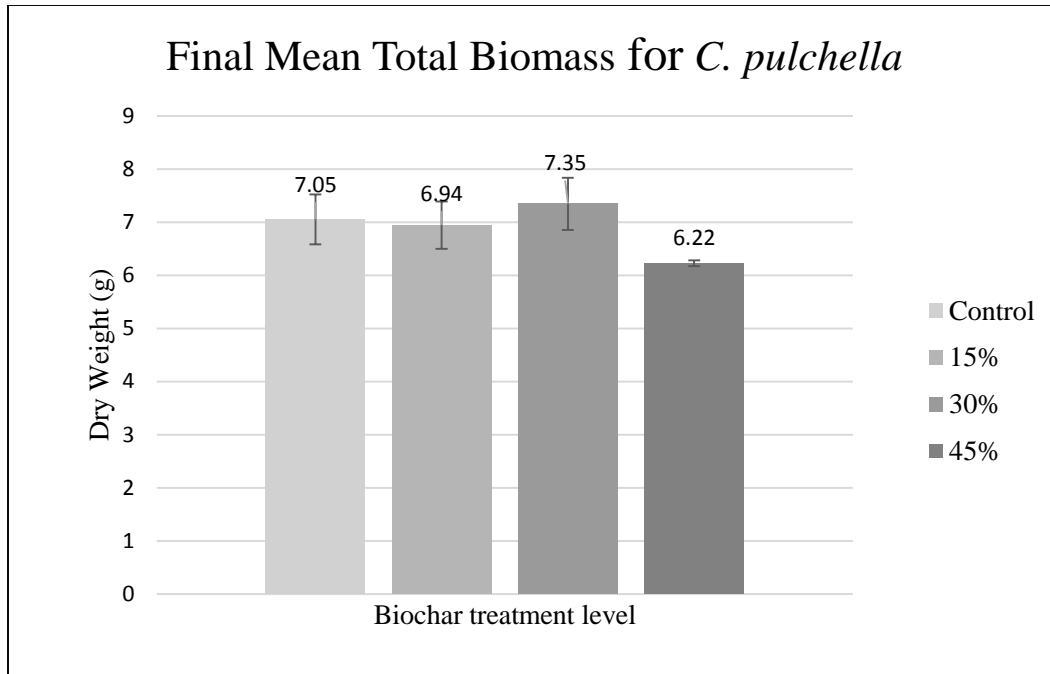


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Figure 6. Mean total biomass accumulation as a function of biochar treatment over time for *P. ponderosa*.

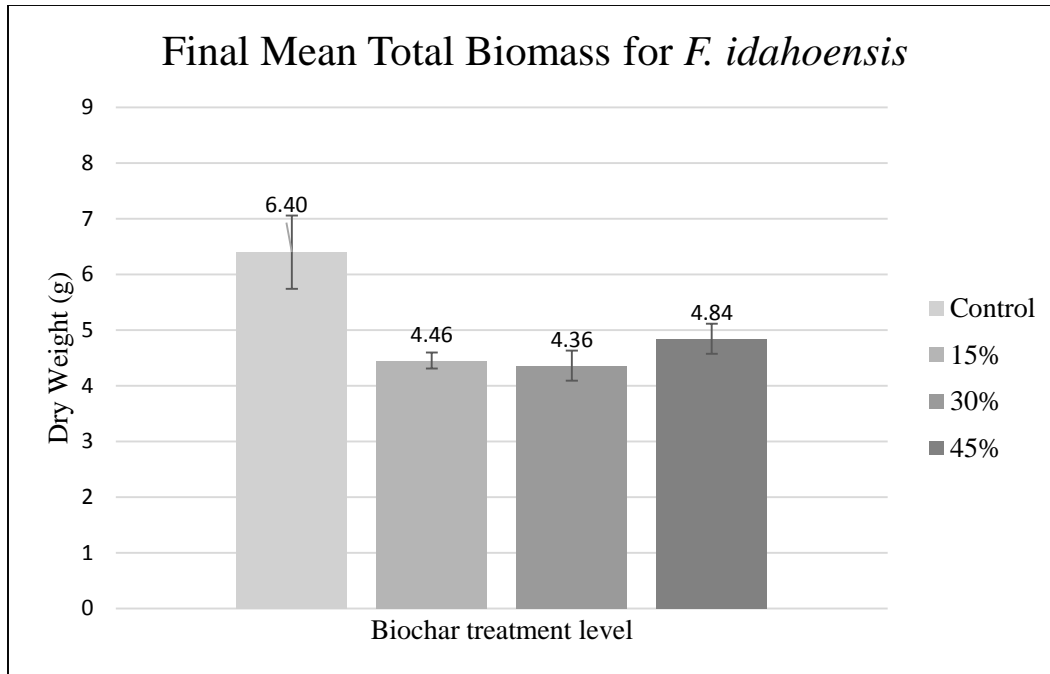


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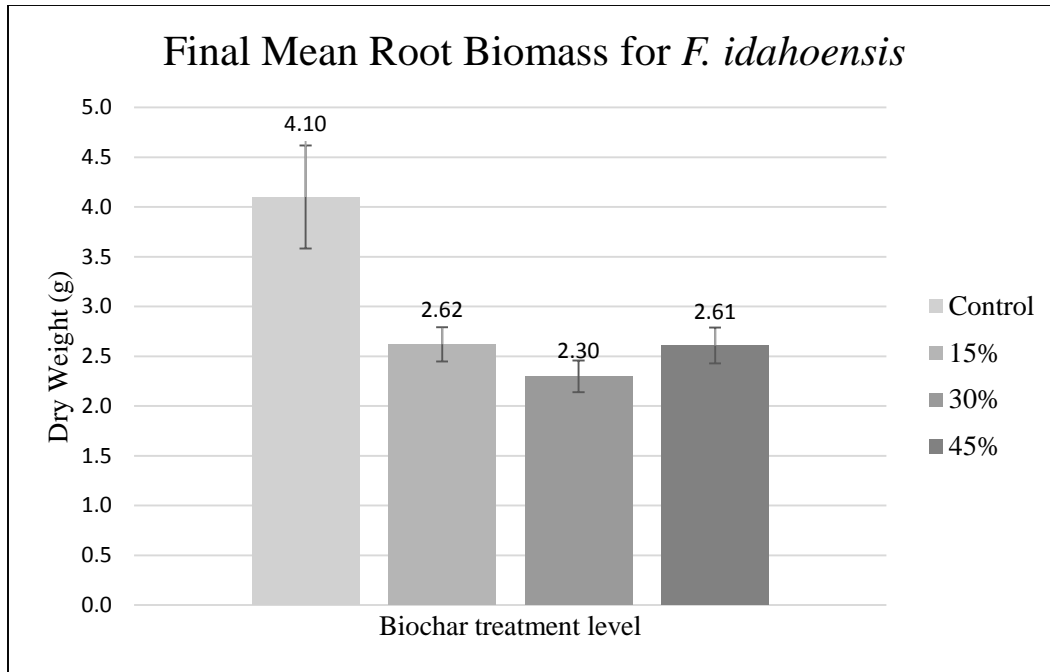
Figure 7. Mean total biomass (dry wt. in grams) as a function of biochar treatments for *C. pulchella*.



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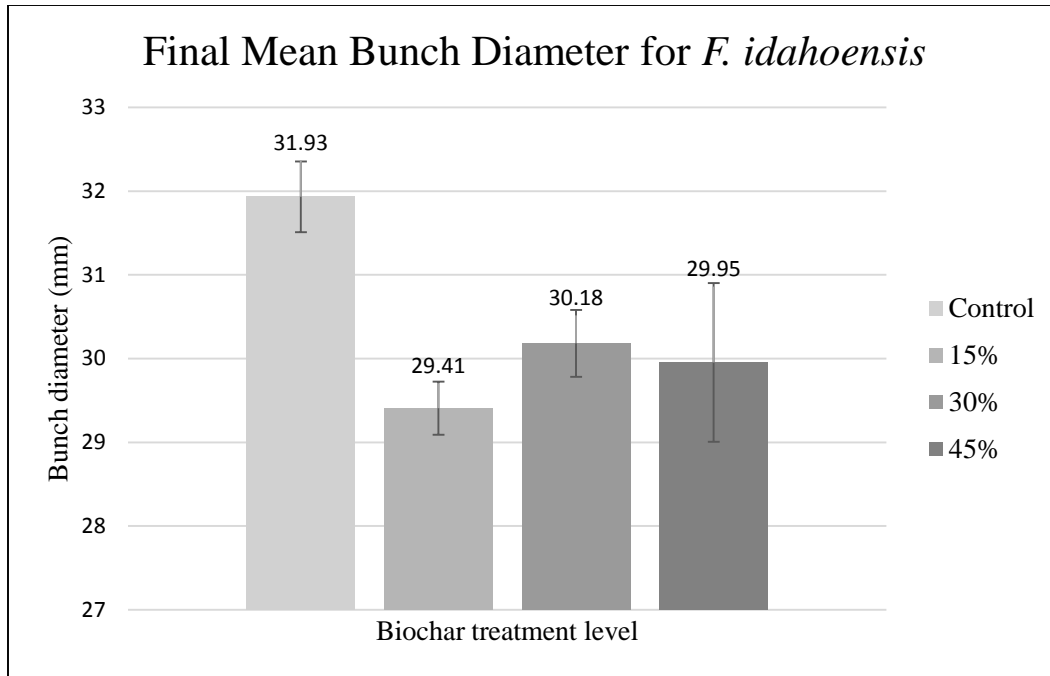
Figure 8. Mean total biomass (dry wt. in grams) as a function of biochar treatment for *F. idahoensis*.



58

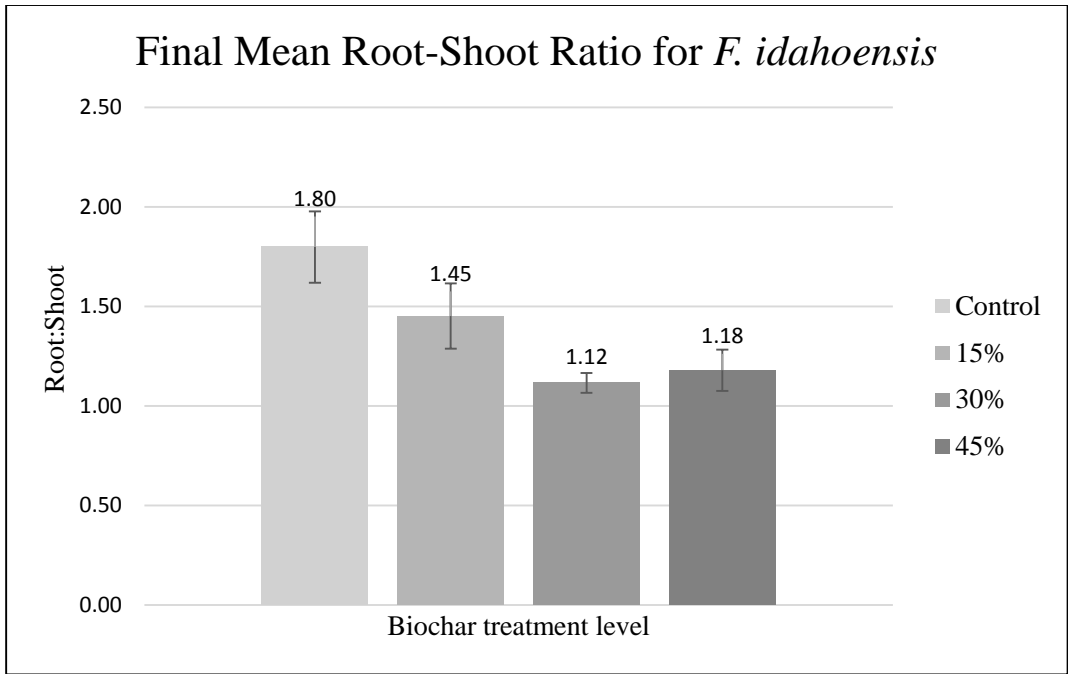
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Figure 9. Final mean root biomass (dry wt. in grams) as a function of biochar treatment for *F. idahoensis*.



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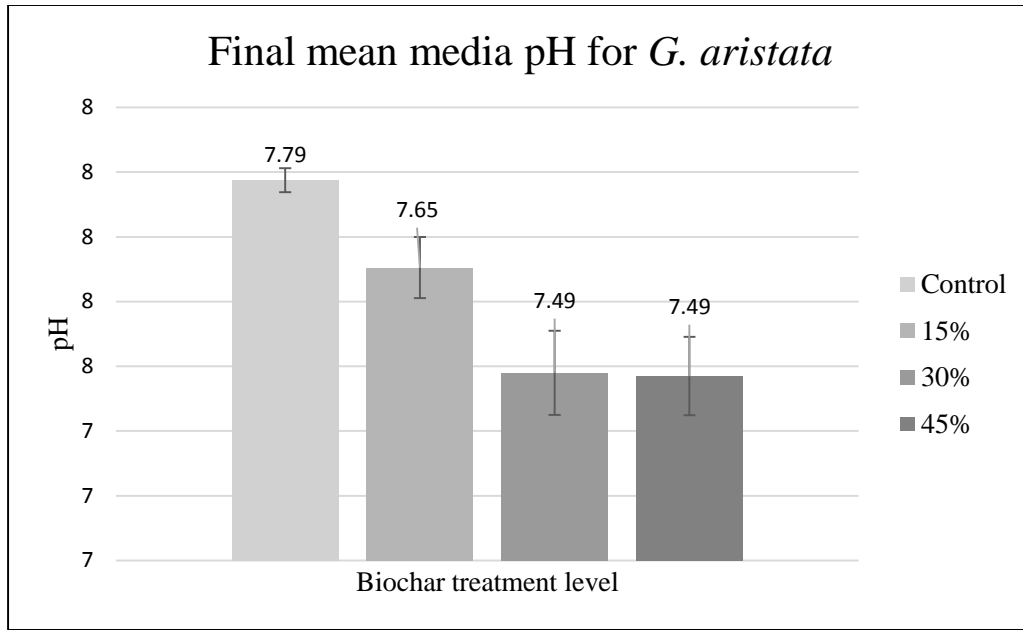
62 **Figure 10.** Final mean bunch diameter (mm) as a function of biochar treatment for *F. idahoensis*.



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64

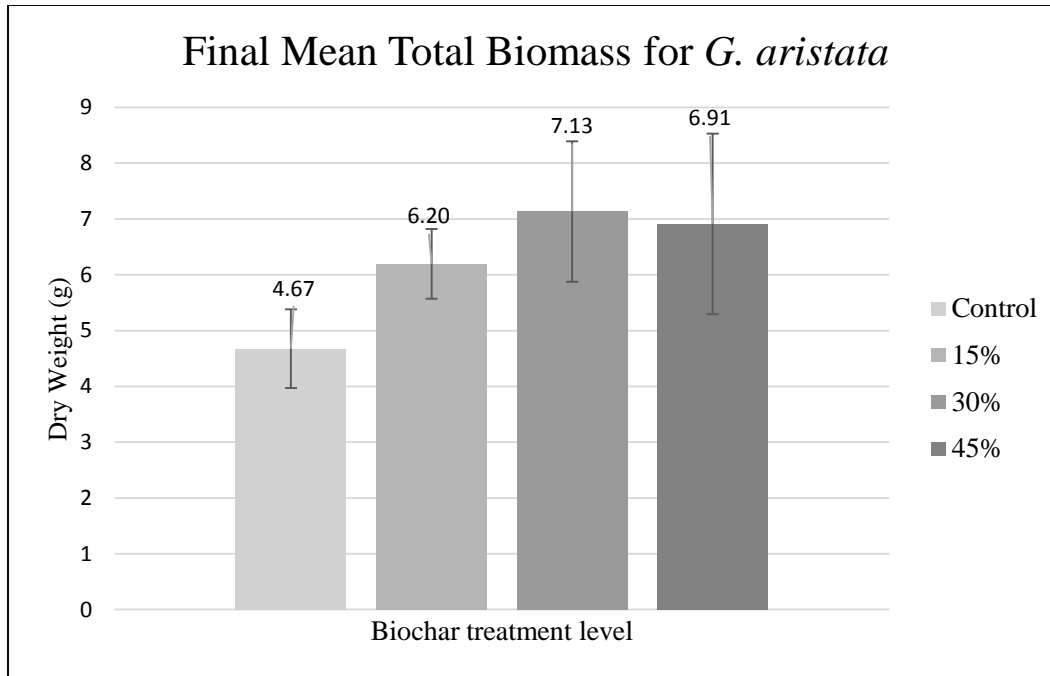
Figure 11. Final mean root-shoot ratio as a function of biochar treatment for *F. idahoensis*.



65

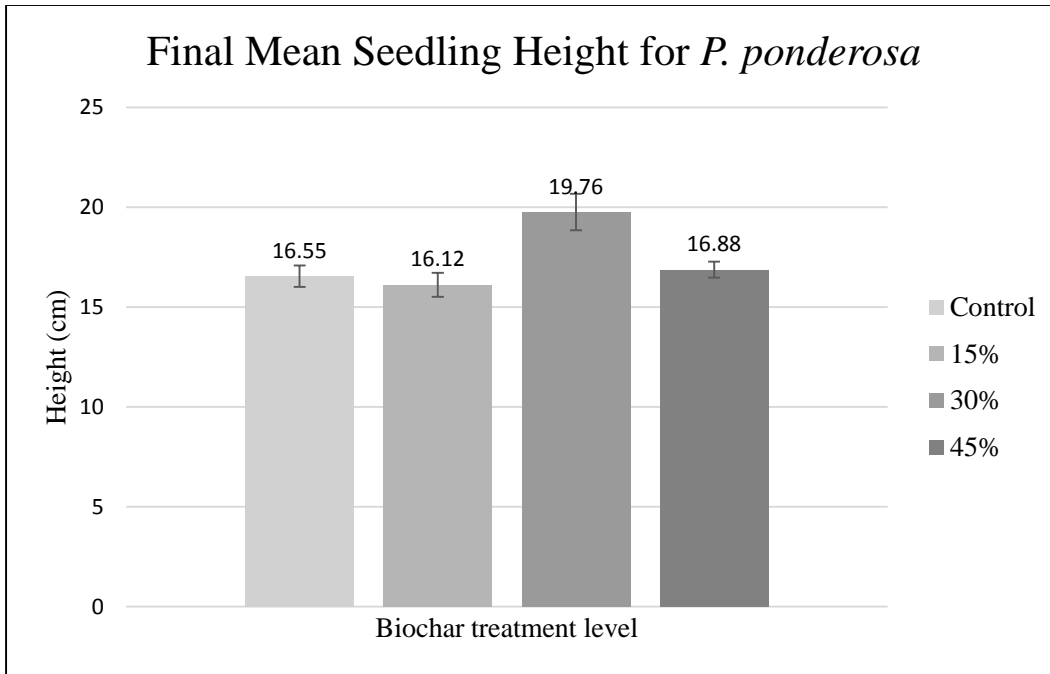
66

Figure 12. Final mean media pH as a function of biochar treatment for *G. aristata*.



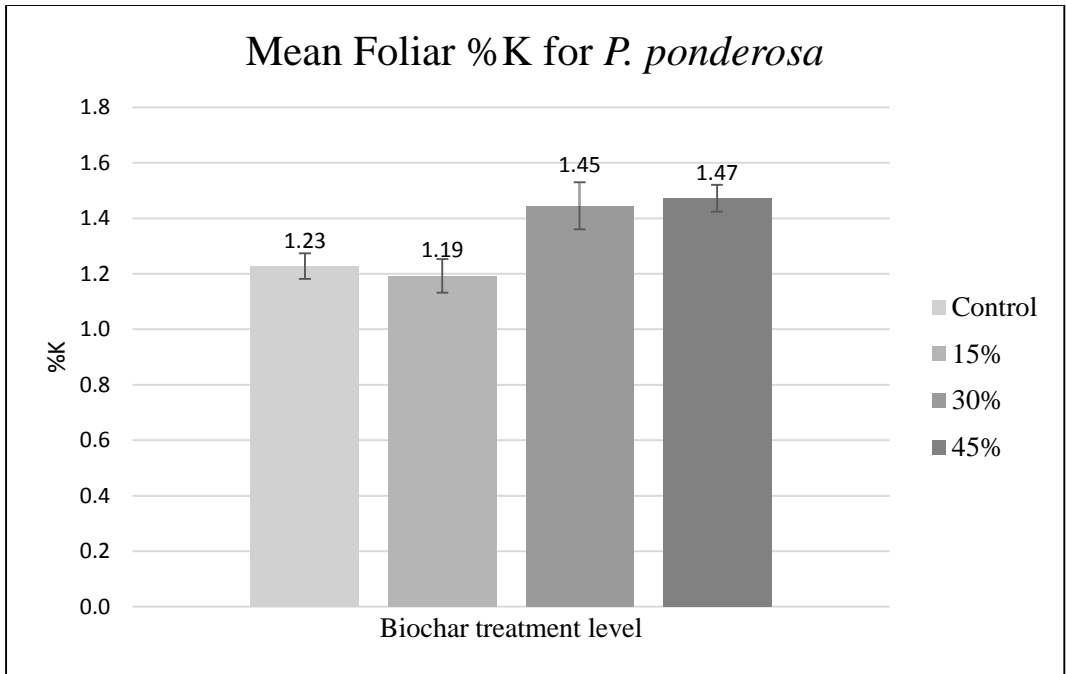
67

68 **Figure 13.** Mean total biomass (dry.wt.in grams) a function of biochar treatments for *G. aristata*.



69

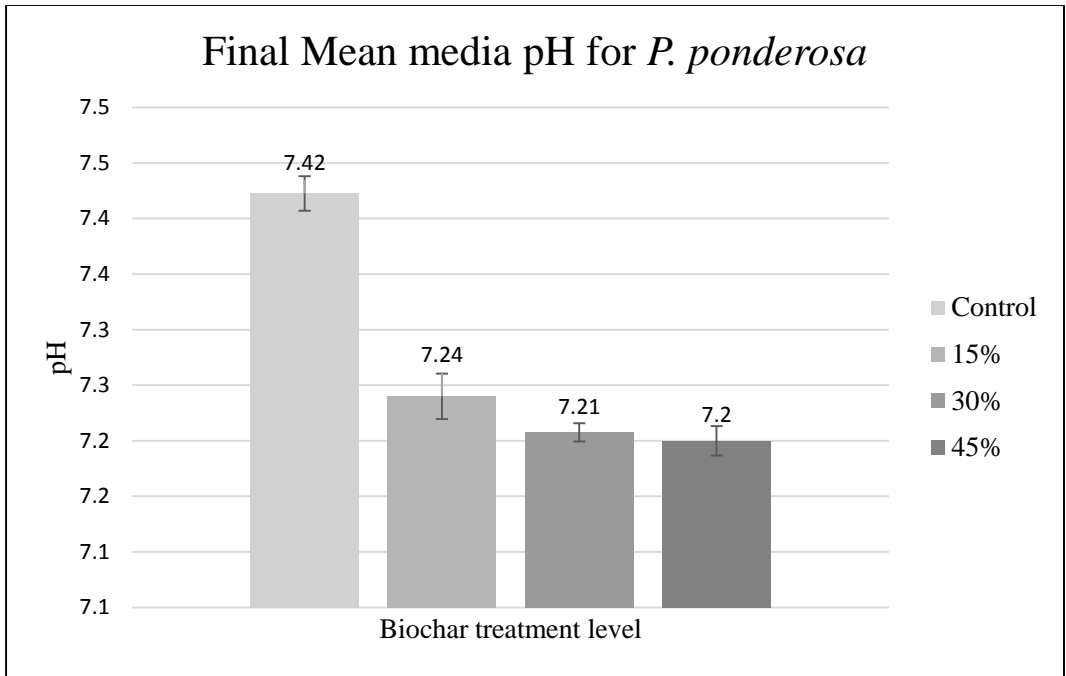
70 **Figure 14.** Final mean seedling height (cm) as a function of biochar treatment for *P. ponderosa*.



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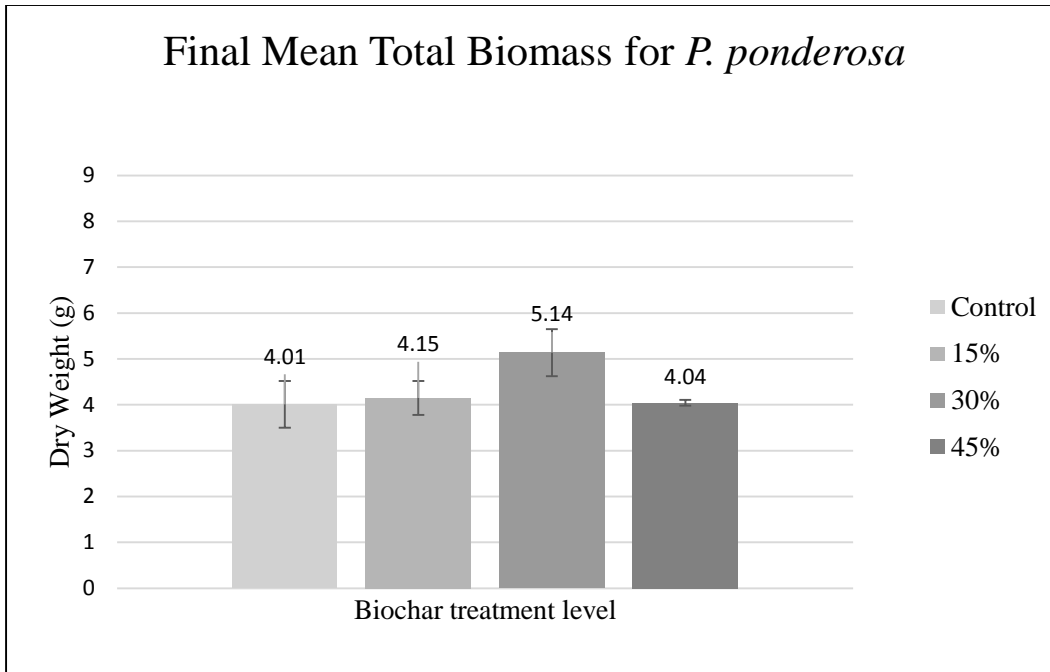
Figure 15. Final mean plant tissue %K as a function of biochar treatment for *P. ponderosa*.



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Figure 16. Final mean media pH as a function of biochar treatment for *P. ponderosa*.



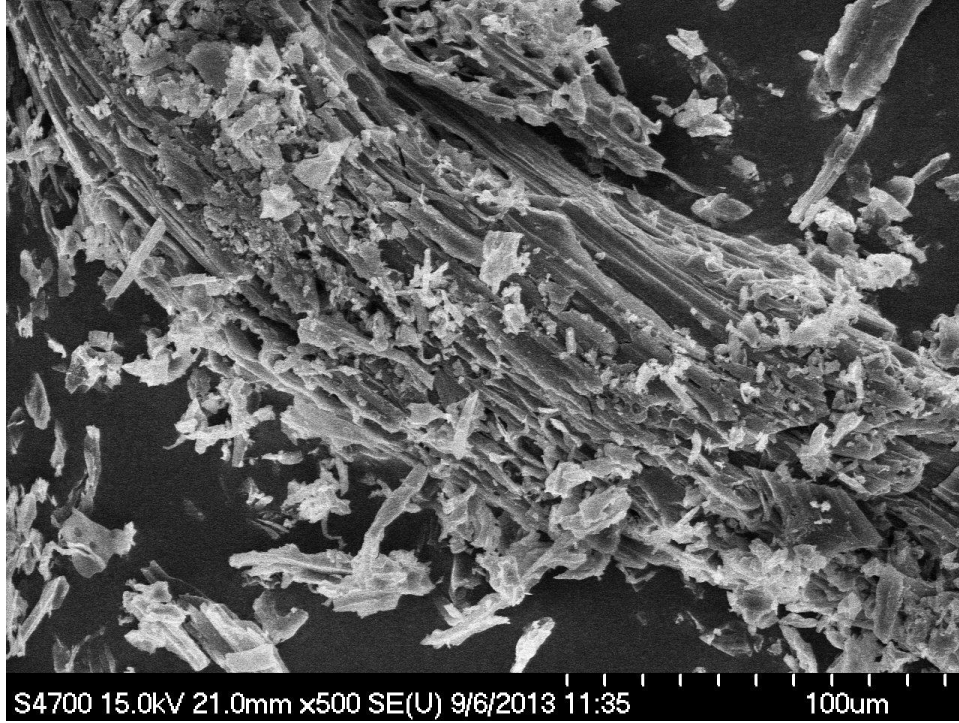
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Figure 17. Mean total biomass (dry wt. in grams) as a function of biochar treatment for *P. ponderosa*.



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Image 1. Electron micrograph of Dynamotive biochar particles under 500x magnification.

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