THE EFFECT OF BIOCHAR APPLICATION AND SOIL TRANSFER ON SURVIVAL AND GROWTH OF TREE SEEDLINGS IN RESTORATION

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Abstract

Harsh conditions hinder the growth and survival of woody plants in restoration of forests and degraded landscapes. Transplanted tree seedlings often desiccate within the first few weeks. Management options to increase survival such as watering or shading may be costly or infeasible. However, low-cost techniques may improve seedling survival by increasing water availability to plants or ameliorating soil conditions. One such restoration technique gaining attention in recent decades is the amendment of soil with biochar. Biochar may increase the moisture retention of the soil, mitigate the effects of soil contaminants, alter soil physico-chemical properties, and may even enhance mycorrhizal fungi colonization of the roots. However, negative effects of biochar have been reported in some agronomic settings. Thus, research is needed before its use in forest restoration can be recommended. Another low cost technique that improves transplant success is soil transfer, intended to inoculate transplanted seedlings with beneficial microbiota and accelerate the establishment mycorrhizal relationships.

To test the potential effects of biochar and forest soil inoculation on tree seedling establishment in a restoration setting, we conducted a systematic review and meta-analysis of the literature in the topic and carried out a field experiment. Results from the meta-analysis of the use of biochar on woody plants in various restoration contexts suggest positive effects of biochar on woody plant growth and survival, with an effect size (ES±SD) of 0.95±0.05 overall, 1.02±0.01 on biomass, and 1.04±0.01 on survival. However, the heterogeneity of biochar production and application—and of restoration context and focal species—prevents broad generalization and indicates the need for additional field studies assessing the effects of biochar on woody plants.

For our field experiments, we transplanted seedlings of northern red oak (*Quercus rubra*) into three disturbed forest areas: a pine plantation ten years post-thinning, a post-plantation recent clear-cut, and an urban forest preserve with an understory cleared of invasive species. Seedlings were planted under four treatments: soil inoculation and biochar (SB), only biochar (BC), only soil inoculation (SI), and control (C). We then monitored seedlings growth and survival. Mortality was high in all treatments and across sites. Our findings suggest that treatments of biochar and soil transfer had no significant influence on *Q. rubra* growth and survival in the first year. In both years, however, the highest positive impact on survival resulted from the SI (-2.09±0.31) and BC (-2.28±0.29) treatments, with the most negative in C (-3.13±0.43). The effect of treatment on average biomass was highest in C (0.43±0.25) and SI± and lowest in BC (-0.10±0.20). Overall, this work contributes to the body of knowledge on the use of biochar and soil transfer in restoration experiments. Use of either or both soil amendment techniques may not be necessary in all systems, and should be tailored to the suit the focal species and ecosystem.

Keywords: biochar, restoration, meta-analysis, seedlings

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Introduction

Under stressful conditions, forest restoration and regeneration may require active intervention. Often, land managers attempt to jumpstart restoration by planting seedlings, but the harsh environmental conditions of some disturbed or degraded forest lands—including lack of moisture, high levels of solar radiation, and low nutrient availability—challenge seedling growth and establishment (Miller 1983; Grossnickle 2012). To facilitate the restoration of forest ecosystems, land managers and landowners with limited resources need simple, inexpensive ways to improve survival of seedlings planted as part of their restoration efforts. Management to ameliorate harsh conditions usually includes modifications made to the physical environment, referred to as ecotechnological approaches (Piñeiro et al. 2013). These may include organic amendments to soil, mycorrhizal inoculations (either directly or through a soil transfer), and the use of nurse shrubs or constructed shelters (Piñeiro et al. 2013). Despite their potential, little research has been done to assess the utility of some of these low cost practices on woody plants (Thomas and Gale 2015; Cho et al. 2017). In this study, we investigate the effects of pyrolyzed biomass (biochar) and local soil transfers (soil inoculations) on tree seedling survival and growth, in areas that have gone through recent thinning, clear cut, or understory clearing. We also conduct a systematic review and meta-analysis of the use of biochar on woody plants in restoration contexts.

Because seed dispersal can limit forest regeneration and because land managers may be interested in moving toward an intended forest community, planting of seedlings is a common restoration practice (Palma and Laurence 2015). The first few years of a seedling represent a crucial window for resource capture and growth, which can determine whether or not a seedling becomes established (Grossnickle 2012). Smaller size classes of trees are subject to

disproportionately high non-random mortality, but recruitment can determine the future structure of the whole community (Perry and Amaranthus 1987; Green et al. 2014). Whether or not a seedling can "couple" with the environmental conditions it is growing under—i.e., successfully begin to absorb water and grow—greatly influences its chance of survival (Grossnickle 2012). Thus, interventions to facilitate this coupling process are desirable in active restoration projects.

Sites that have been deforested, harvested, or disturbed may have increased levels of solar irradiation, fewer nutrients as a result of leaching, less soil biodiversity, and other constraints on seedling survival (Oliet and Jacobs 2012; Jacobs et al. 2015; Mahendrappa et al. 1986). Highly degraded sites, such as reclaimed mine lands, face even greater barriers (Oliet and Jacobs 2012). Disturbed sites may also become resistant to restoration efforts, as a result of changes in connectivity of forest habitats, the introduction of nonnative species, and changes to biogeochemistry of the site (Suding et al. 2004). Management practices that ameliorate these stressors can be essential to ensure the success of the restoration. The transplant of soil from intact plant communities and the application of biochar may be two potential strategies that managers can use in the context of restoration or intervention ecology.

Soil inoculation, or soil transfer, involves the transplantation of a relatively small amount of soil. Evidence suggests that soil communities may influence the establishment of plant communities (Wubs et al. 2016). Experiments conducted in grasslands demonstrate that the introduction of native soil communities through soil transfer can not only increase the soil faunal diversity but potentially facilitate the assembly of species towards a "target ecosystem," such as grassland or heathland (van der Bij et al. 2018; see also Wubs et al. 2016). For restoration with woody plants, the introduction of mycorrhizal fungi spores is often the primary goal of soil transfer experiments. Mycorrhizal fungi are below-ground symbionts that associate with more

than 90% of all vascular plants (Aerts 2003; Read 1997). They facilitate seedling survival via enhanced nutrient and water absorption, as well physical protection from pathogens (Harley and Smith 1983; Botton and Chalot 1999; Hawkins et al. 2015). Two broad categories of mycorrhizae are most relevant to restoration of woody plants: arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) (Finlay 2008). In particular, EMF fungi can affect early establishment during field plantings, as the fungal sheath they form around the root dramatically increases contact with the soil and can thus significantly affect the nutrient status of the plant (Simard et al. 2003). Mycorrhizal networks that connect seedlings to other plants, including mature trees, can also facilitate survival during the challenging establishment phase for seedlings by providing carbon transfers (Bingham & Simard 2011). The composition of the mycorrhizal community has been show to affect the success of EMF seedlings (e.g., O'Brien et al. 2010), and can increase plant species richness and facilitate the transition toward target or reference ecosystems (Neuenkamp et al. 2017).

Nursery-grown seedlings can be inoculated with commercial mycorrhizal species, but this is costly (Vosatka and Dodd 2002). Furthermore, the organisms in these products might not be reflective of the natural mycorrhizal fungi community at that site of the restoration (Vosatka and Dodd 2002). Deforested or badly degraded lands may have reduced abundance and diversity of mycorrhizal fungi, but soil transferred from intact or reference communities may contain fragments of the mycelial network (Asmelash et al. 2016; Read 2002). Introducing soil from a local forest with adult trees of the planted species may facilitate the seedling-mycorrhizal association and may infer higher survival to the plant. For example, Amaranthus and Perry (1987) observed 50% increases in survival of Douglas-fir (*Pseudotsuga menziesii* Mirbel) seedlings when soil from an intact plantation was applied to plantings in a clear cut. However, a

more recent study failed to demonstrate a positive effect of soil inoculation on Douglas-fir seedling survival (Grove et al. 2019). Limited research on other woody plants is available.

Soil amendment with biochar is another low-cost practice that may enhance seedling establishment. Biochar refers to charcoal formed by burning materials such as wood or leaves for use as a soil amendment (Lehmann and Joseph 2009). More technically, biochar is a C-rich solid that is formed during the pyrolysis (thermal decomposition) of biomass in the absence of oxygen at high heat (Lehmann and Joseph 2009). It primarily consists of polycyclic aromatic hydrocarbons, which can resist decay in the soil for years (Atkinson et al., 2010; Lehmann and Joseph 2009; Glaser et al., 2009). Soil amendment with biochar has been associated with small positive increases plant growth and aboveground productivity (Biederman and Harpole 2013; Atkinson et al. 2010; Jeffery et al. 2011). Thus far, its primary application has been in the agricultural industry (Jeffery et al. 2011; Thomas and Gale 2015). However, in the last decade, it has also been applied by foresters and restoration ecologists to promote growth and survival of woody plants (Thomas & Gale 2015). A recent meta-analysis examining the effects of biochar on woody plants found an average increase in biomass of 41% (Thomas & Gale 2015). Because of the recalcitrance of the C-containing compounds in biochar and because it can be sourced from waste products, it is sometimes hailed as a "sustainable" C sequestration mechanism (Glaser et al. 2009; Woolf et al. 2010). In addition to soil improvement and C sequestration, energy production can occur during the biochar pyrolysis (Lehmann 2007). With the potential to re-use waste, generate energy, and stimulate agricultural or forest productivity, some have enthusiastically labeled it a "win-win-win" (Biederman and Harpole 2013).

There are several mechanisms by which biochar might favorably alter the physical environment for tree seedlings. First, biochar contains soluble nutrients, increases water

retention, and can even increase soil pH (Thomas & Gale 2015). Biochar differs from soil in several key physical and structural characteristics, including hydrodynamics, pH, cation exchange capacity (CEC), tensile strength, soil bulk density, surface area, and gas exchange (Lehmann et al. 2011; Major et al. 2010; Atkinson et al. 2010). Because biochar particles have negative surface charge and large surface areas, soil amendment with biochar may decrease leaching of nutrients and thus increase their availability for uptake by plants (Noyce et al. 2017; Atkinson et al. 2010; Laird et al. 2010; Biederman and Harpole 2013). Finally, due to its porous structure and ability to retain water, use of biochar can improve water retention in a variety of soils, including sandy soils and clay soils (e.g., Bruun et al. 2014; Sun & Lu 2013; Abel et al. 2013; Obia et al. 2016). However, other studies have demonstrated no effect of biochar on soil porosity and moisture content (e.g., Hardie et al., 2014). The effects of biochar may depend on soil type (Spokas et al. 2012). The greatest positive effects on crop growth in a meta-analysis by Jeffery et al. (2011) were observed in acidic or neutral soils, with coarse or medium texture. Different vegetation may also be more or less responsive to biochar soil amendment—for example, evidence suggests conifers may be less responsive than angiosperms (Thomas and Gale 2015; Noyce et al. 2017).

Some studies have shown the addition of biochar to soil to be beneficial to mycorrhizae (Warnock et al. 2007; Solaiman et al. 2010; Hammer et al. 2014). There are several possible explanations for this. Biochar alters soil properties, influences mycorrhizal relationships with free-living microbes, and adsorbs harmful allelopathic chemicals from the soil (Warnock et al. 2007; Jaafar 2014). The porous structure of biochar may also provide refugia from grazing soil organisms for either fungal hyphae or beneficial bacteria, such as mycorrhizal helper bacteria (MHB), which exude metabolites that promote the growth of hyphae (Warnock et al. 2007;

Lehmann et al. 2011). Biochar, by altering soil nutrient availability, could also shape the relationship between plants and fungi. In high soil nutrient levels—e.g., as in a greenhouse—the symbiosis between plant and fungi may shift from mutualism to parasitism for the plant (Jones and Smith 2004; Johnson et al. 1997). Some studies have found decreases in mycorrhizal colonization with increasing dosage of biochar (e.g., Budi and Setyaningsih 2013), perhaps because of altered nutrient availability following biochar amendment.

Finally, due to its sorptive properties, biochar may alter chemical signaling between plants, microbes, and fungi by serving as a sink for these signaling compounds (Warnock et al. 2007). It has also been shown to mitigate the negative effects of allelopathic chemicals from plants (Sujeeun and Thomas 2017) and to be capable of sorption of pollutants such as herbicides (e.g., Wang et al. 2010). The sorption of inhibitory alleleopathic chemicals might benefit mycorrhizae, but the sorption of chemicals produced by plants to promote colonization or fungal branching could decrease association (Warnock et al. 2007). Similarly, sorption by biochar may remove contaminants that inhibit microbial abundance (see Lehmann et al. 2011 for further discussion of interactions with soil microbiota). It is important to note that biochar made from different feedstocks and under different pyrolysis conditions can vary immensely (Enders et al. 2012; Thomas & Gale 2015). In light of this, it is likely that the structure, chemical properties, pore size, and other characteristics of any given biochar may shape both the directionality and magnitude of any of the interactions described above.

If amendment with biochar or soil from intact plant communities increases soil water retention or nutrient availability, these interventions may prove to be useful additions to the restorationist's toolbox, reducing the need for manual watering or use of synthetic fertilizers. Given the variability of results found across soil inoculations and biochar additions, research on

the use of biochar on tree seedlings in temperate climates is needed before the treatment is applied at larger scales. To understand the effects of biochar, we conducted a systematic review and meta-analysis of its effects on the growth and survival of woody plants in the context of restoration. Next, across two field seasons and in three locations in Michigan, USA, we sought to examine the effects of both applying soil from a nearby hardwood forest (as an inoculum of microbiota or as a "natural" dose of fertilizer) and of biochar to planted tree seedlings. Specifically, the experiments address two questions:

1. Does transfer of soil from proximate, "high-quality" hardwood forests increase seedling growth and survival in the first season?
2. Does the use of biochar increase seedling growth and survival in the first season?

Although there is evidence that each of these techniques may be beneficial in restoration projects, there is a gap in the data regarding: a) their use in woody plant restoration; b) their use in combination with one another. Findings from this research were aimed at aiding managers in the identification of low-cost, easily applied treatments that might facilitate transitions from disturbed area to native forested communities.

Meta-analysis

Literature search

In October of 2019, systematic searches were conducted in two databases: Web of Science (Core Collection) and Scopus. Only peer-reviewed articles written in English were included. In addition to these two databases, the first 150 returns on Google Scholar (using shortened search terms) were also reviewed. (See Appendix A for full search terms for each database.)

In all, we screened 608 search returns for relevancy based on title and abstract. Specifically, studies needed to be focused on seedlings of woody plants or trees, ecosystem restoration, and biochar application. Studies focused on agricultural plants (such as *Prunus* spp. or *Malus* spp.) were excluded. Studies reporting only changes to soil (e.g., nutrient levels) were excluded. To reduce the heterogeneity of studies, we also excluded those based on other pyrolyzed wood products (e.g., wood ash, wood vinegar, and biochar pellets) and those studies focused on hearth or 'Terra Preta' soil, in which the charred material was burned decades ago, in indeterminate conditions. Some studies compared both biochar application and wood ash application to control, but in these cases we only extracted data related to biochar. When multiple levels of biochar application were present, we included data on the highest and lowest application rate. If studies examined multiple fertilization regimes, we used only data from the lowest fertilization application. After duplicate removal and selection criteria were apply we ended with 26 peer-reviewed articles that we included in the meta-analysis (Appendix B). In addition to these, three review papers identified in the systematic review were reviewed in detail (Stavi, 2013; Thomas and Gale 2015; Biederman and Harpole 2013).

Data extraction and effect size calculation

From the articles that met inclusion criteria, we extracted data on location, ecosystem type, soil type, plant species, and biochar characteristics (including pyrolysis temperature, feedstock, and application rate). When pH of biochar was measured with CaCl2, we used the formula pH-H2O = 1.65 + (0.86*[pH-CaCl2]), as per Biederman and Harpole (2013). Main categories of response variables measured were survival, shoot/root ratio, height, diameter, root volume, growth rate, germination, biomass, and mycorrhizal colonization.

To measure effect size, we used the natural log-transformed response ratio: ES = ln (T/C). In this case, T is the measured value of the response variable and C is the measured value of the control (i.e., no amendment with biochar). To estimate each observation's ES, mean and SD, we ran a bootstrap (10000 iterations) randomly drawing values from the reported treatment and control means and their associated variability. Sample sizes were also accounted by weighing the reported variances by the sample size (Gurevitch and Hedges 1999).

Data analysis

We analyzed ES in three different ways all following a hierarchical structure where we estimated ES for different groups nested within an overall ES estimate. The groupings we analyzed were: for each type of response found in the data (including study random effects), for each genus represented in the data, and for each study (publication) included. We did not include study as random effect in the genus level analysis because most genera were only represented in one study. There was a small number of observations (N=30), that did not report variance around the control and/or treatment response. For those, we considered these missing variances as latent variables that were estimated as function of the largest ES variance calculated from observations with reported variances (Batson and Burton, 2016). We sampled from normal distributions

(limited to be positive) with estimated largest variance as the mean and a SD of 1. For observation *i* with effect size mean ES_i and standard deviation σ_i:

$$ES_i \sim \text{Normal}(ES_{\text{group(i)}}, \sigma_i^2)$$

When study random effects (SRE) were included, we used:

$$ES_i \sim \text{Normal}(ES_{\text{group(i)}} + SRE_{\text{study(i)}}, \sigma_i^2)$$

Due to the latent missing variables, parameters were then estimated following a Bayesian approach with non-informative prior distributions:

$$ES_{\text{group}} \sim \text{Normal}(ES_{\text{overall}}, \sigma_{\text{overall}}^2)$$

$$ES_{\text{overall}} \sim \text{Normal}(0,100),\ SRE_* \sim \text{Normal}(0, \sigma_{SRE}^2),\ \text{and}\ \sigma_*^2 \sim \text{Uniform}(0,100)$$

Analyses were performed in OpenBUGS (Thomas, 2006; see Appendix C for analysis code).

Field experiment

Study sites

The field experiment took place at two latitudes in Michigan (Fig. 1). The experiment was carried out in the summer of 2018 at the northern sites , and in 2019 at the southern site. The area has a humid continental climate, averaging 6.2ºC in temperature annually, with around 965 mm of precipitation per year on average (NOAA, NWSFO, Gaylord, MI). The first northern site was situated part of the Little Traverse Conservancy (LTC) and it is situated near Harbor Springs, MI (45.4497° N, 84.9253° W), this is a red pine plantation that it is being transitioned to native hardwood forest (Fig. 2). The site was selected because it is representative of many areas that have been heavily managed or disturbed by human use in the past, and because it is the goal of the LTC to cease active management of the red pine plantation and transition to a mixed-hardwood forest. The second northern site was situated in a clear-cut section of forest at the University of Michigan Biological Station (UMBS) near Pellston, MI (45.553889° N, 84.784444° W). The site was clear cut in fall of 2017 (Fig. 3). The southern site was at the Nichols Arboretum, a property of the University of Michigan, in Ann Arbor, Michigan, USA (42.2810° N, 83.7256° W). The area has a humid continental climate, averaging 9.3°C annually, with around 818 mm of precipitation per year on average (NOAA, NWSFO, Ann Arbor, MI, USA). The Arboretum is currently intensively managed for invasive plant species, particularly shrubs such as buckthorn and honeysuckle, using both physical and chemical treatments. Although managers are not currently restocking the forest with seedlings, the urban location and combination of stressors (such as invasive plants) make it an interesting location to examine the effects of biochar and soil transplant on seedling growth and survival.

Plantings and treatments

We planted northern red oak seedlings (*Quercus rubra*, Linn.*).* As a moderately high light-demanding species, these oaks may benefit from thinning and/or burning treatments in transitioning plantations (Loftis 1983). *Quercus rubra* grows quickly and has an intermediate tolerant for shade, which made it a suitable candidate for planting in the open spaces of the pine plantation (Barnes & Wagner, 2004). *Quercus rubra* seeds were a mixture of wild seeds from Pennsylvania and Michigan (see Appendix D). The seeds were stratified and germinated in the greenhouse in potting soil, a blend of peat, bark, and perlite (Metro Mix 830, Sun Gro Horticulture, MA, USA).

For the northern 2018 plantings, germinated seeds were grown for 4-5 weeks in tubs with potting soil. Treatments were applied to bare-root seedlings at the time of planting. For the soil inoculation, soil was collected from a nearby hardwood forest community. Because there were no adult Q. rubra in the adjacent community to LTC, soil was collected 2-3 m away from the base of adult beech (*Fagus grandifolia* Ehrh.), paper birch (*Betula papyrifera* Marshall), or yellow birch (*Betula alleghaniensis* Britt.), as members of the family Betulaceae and Fagaceae are EMF hosts (Ishida et al. 2007). At UMBS, soil was collected from beneath adult *Q. rubra*. The soil was collected from the top 10-20 cm of soil, sieved at 2 mm, homogenized, and placed in the hole into which seedlings were planted. The biochar was sold commercially as “pure granular biochar” made from a “yellow pine” feedstock, with a pH of 7.4 (Wakefield Agricultural Carbon, MO, USA). Additional characteristics of the biochar can be found in Appendix E. A volume of 150 mL of biochar was applied to the hole as each seedling was transplanted. Seedlings were divided in four groups, control (C) no additions, biochar (BC) added, soil inoculum (SI) added, and soil inoculum and biochar (SB) added. Fifteen seedlings

per treatment were planted at each of three 2 x 2 plots per site (3 plots x 4 treatments x 15 replicates per treatment, N = 180 per site). Plots were located in the space between rows of planted red pine (*Pinus resinosa* Sol.) trees at LTC (Fig. 2) and in the clear cut area of UMBS (Fig. 3). To account for maternal effects in seedling source, seedling height was measured before transplant. Seedling planting occurred on 6 Jun 2018 and harvest occurred on 29 August 2018, before senescence. Colonization by EMF was assessed in the surviving seedlings from the 2018 plantings. Soil and debris was removed from the roots of seedlings using deionized water. Percent EMF colonization was determined by counting the number of colonized tips out of 100 root hairs, selected randomly from the root mass, using a dissecting microscope at a magnification of 200X (Perry et al. 1989; Grove et al. 2019). In some cases, there were fewer than 100 viable root hairs for analysis. In these instances, all viable root hairs were analyzed. Percent EMF colonization was estimated as the percentage of root tips with EMF (visible mantle of hyphae on the root) out of total number of tips observed.

For the 2019 southern site plantings, soil inoculation occurred during greenhouse planting. Soil was collected from a nearby hardwood forest community, at Radrick Forest, a 35-acre upland oak-hickory forest near Ann Arbor, MI (Hammitt and Barnes, 1989). Following the same protocol described above, at planting, 150 mL of soil inoculum or/and biochar was mixed into individual seedling containers (0.65L) with potting soil (Metro Mix 830, Sun Gro Horticulture, MA, USA), resulting in in approximately 25% v/v. Seedling height was measured right before planting in the field, approximately 4-5 weeks after germination. At this site, two 2 x 2 m plots were set up at three locations. Each plot contained the four treatments, with 12 seedlings per treatment and 60 seedlings per plot, for a total of 288 seedlings (3 paired plots x 4 treatments x 12 replicates per treatment; N = 288). In each plot, one of the four treatments was

randomly assigned to one subplot. Seedling planting occurred on 8 June 2019 and harvest occurred on 8 September 2019, before senescence.

After harvest, seedlings were dried for 24 hours in an oven at constant temperature of 75ºC to remove moisture. Biomass of dried seedlings was measured by weighing the leaves, stem, and roots of the plant separately.

Data analysis

All response variables, survival (0 dead, 1 alive), biomass (g) and mycorrhizal colonization (%; only for LTC) were analyzed as a function of treatment, plant size at the time of planting (to account for maternal effects, since this factor could affect the actual responses to treatments) and of plot random effects (PRE; nine total, three per site) for survival or of site random effects (SRE; three total) for biomass and mycorrhizae (because there were not enough surviving seedlings to use plot random effects). For each seedling *i* we analyzed the data with the following likelihoods and process models:

$$\text{survival}_i \sim \text{Bernoulli}(p_i),\ \text{logit}(p_i) = \alpha p_{\text{treatment(i)}} + \beta p \cdot \text{Plantedheight}_i + \text{PRE}_{\text{plot(i)}}$$

$$\text{biomass}_i \sim \text{logNormal}(b_i, \sigma^2),\ b_i = \alpha b_{\text{treatment(i)}} + \beta b \cdot \text{Plantedheight}_i + \text{SREb}_{\text{plot(i)}}$$

$$\text{mycor}_i \sim \text{Poisson}(m_i),\ \ln(m_i) = \alpha m_{\text{treatment(i)}} + \beta m \cdot \text{Plantedheight}_i + \text{SREm}_{\text{plot(i)}}$$

To estimate parameter values, we used a Bayesian framework with non-informative prior distributions, $\alpha^*, \beta^* \sim \text{Normal}(0,1000)$, α_m logNormal(1,1000), PRE or $\text{SRE}_* \sim \text{Normal}(0, \sigma_*^2)$, and $\sigma_*^2 \sim \text{Uniform}(0,100)$. Analyses were performed in OpenBugs 3.23 (Thomas 2006). Parameter values, posterior mean, 95% credible intervals, and standard were estimated from 50,000 iterations (see full model code, Appendix F).

Results

Meta-analysis

The locations of the 26 experiments were skewed in geographic distribution, with most studies in Australia (N = 6), Canada (N = 5), and the USA (N = 4), and the remainder in Brazil (N = 2), Indonesia (N = 2), Republic of Korea (N = 2), and with one experiment each from Laos, Finland, Sweden, Nigeria, and Peru (Appendix B). Most of the experiments took place in in temperate (42.31%), tropical climates (26.92%), and boreal (23.08%) climates, with the remaining two experiments in arid and Mediterranean climates. Biochar feedstock was most commonly woody plant material (68.29%), with agricultural wastes, peat, grasses, and other various biomass comprising the remaining feedstocks—including a few surprising feedstocks, such as crab shells (Appendix B).

In all, the meta-analysis involved 378 studies, with an average effect size (ES)±SD of 0.95±0.05. For all response variables measured, the ES was significantly positive (Fig. 4). The ES of biomass (1.02±0.01, N = 171) was greater than the ES of survival (0.86±0.002, N = 19). However, when analyzed at the genus level (N = 31, plus one level of "multiple species"), the overall ES±SD was 0.02±0.05 and the results across genera were mixed (Fig. 5). The genus Acer had the highest positive ES (0.59±0.02), while the genus Aquilaria had the highest negative ES (-0.72±0.01). Biochar application had a small but significant negative effect on Quercus seedlings (-0.07±0.006). Only four of the 26 genera included in the reviewed studies were gymnosperms. Biochar application had a significant negative impact on Juniperus (-0.08±0.007) and Picea (-0.02±0.00006), a significant positive impact on Pinus (0.41±0.05), and no significant impact on Pseudotsuga (Fig. 5).

Field experiment

Overall, observed survival was low across treatments in both years, thus sample sizes for biomass and mycorrhizal fungi colonization (2018 LTC seedlings only) were low (Table 1). Parameter estimates for all the analyses are presented in Appendix G.

Across both years, the SI and BC treatments had the highest positive impact on survival, but the differences between treatments were not significant (Fig. 6). The effect of initial seedling height on survival was not significant (β parameter mean ± SD: 0.22±0.14). Across both years, the C treatment had the highest positive impact on biomass, while the BC treatment had the lowest, but the differences were not significant (Fig. 7). The effect of initial seedling height on biomass was not significant (β parameter mean ± SD: 0.13±0.44). Predicted mycorrhizal colonization was greatest in C and lowest in SB. The EMF colonization model had a low R^2 (0.07) and failed to predict colonization rates at harvest accurately. For this reason, mycorrhizal colonization assessment was not repeated in 2019. All parameter values are reported in the Appendix G.

Discussion

Restoration of ecosystems is a crucial part of conservation and sustainable land management in the 21st century (Hobbs & Harris 2001). Forest loss is often rapid, and the reestablishment of forests can take decades (Chazdon et al. 2016). Although techniques to facilitate seedling growth and survival, such as the soil transfer and the use of biochar have been explored in agricultural and grassland restoration contexts, fewer studies have investigated these ecotechnological approaches in woody plants (Thomas & Gale 2015). Planting seedlings like the oaks in our study may represent either restoration efforts (which strive to restore native species and emphasize ecological integrity) or rehabilitation efforts (which may involve non-native species and emphasize recovery of ecological function) (Chazdon et al. 2016). In our study, the effect of biochar, soil transfer, or the combination treatment had no significant influence on *Q. rubra* seedling survival and biomass, and thus we cannot recommend their use to practitioners in similar contexts without additional research.

As other reviews have found, however, our meta-analysis suggests the effects of biochar on woody plant growth is generally positive (see Biederman and Harpole 2013; Thomas and Gale 2015). In contrast with the findings of our study, the effects were especially high on seedling diameter (ES±SD, 1.04±0.01) and biomass (1.02±0.1; see Fig. 4). In a study of multiple soil amendments on *Acer saccharum* (Marsh.) and *Gleditsia triacanthos*, biochar increased growth 44% compared with control, across both species and in three different soil types (Scharenbroch et al. 2014). Several of the studies in the review found no significant effects of biochar on survival (*e.g,* de Farias et al. 2016). Others reported increases growth only in combination with other treatments. For example, a combination of biochar plus cattle manure increased biomass by 26% compared to control (Lima et al., 2015), and significantly increased

growth in combination with NPK fertilizer but not without (Fagbenro et al. 2013). Biochar production method and feedstock may also greatly influence plant responses. The meta-analysis by Biederman and Harpole (2013) found that high temperature biochars were more alkaline and thus had greater positive effects on aboveground productivity, with overall ES of biochar increasing from slightly negative to strongly positive as pH increases. In some studies in our meta-analysis, biochar promoted the growth of non-target species or even reduced seedling growth. For example, Bieser and Thomas (2019) found significant increases in growth of non-target species such as *Rubus idaeus* and *Solidago canadensis* after biochar application. Aung et al. (2018) found that biochar application decreased aboveground biomass of seedlings compared to control. However it increased the quality index of the seedlings by 14.1% (Aung et al. 2018). Differences in growth and survival in different species at the same site were also reported. For example, biochar applied to saline soils in Australia significantly increased the height of *Eucalyptus viminalis* by 5.1 cm in highly saline soil compared to control treatments, but had no significant effect on *Acacia mearnsii* or in other soil conditions (Drake et al., 2016). Other studies noted increased in soil nutrient availability (*e.g.,* P in biomass increased 30-50% relative to controls at different application rates) and on plant nutritional status, despite neutral effects on growth (*e.g.,* Reverchon et al. 2015; Drake et al. 2016). In the first known study to assess the effects of biochar on reforestation using direct seeding Drake et al. (2016) found increases in the diversity of germinants from a seed mix as well as increases in soil C, N, and P. The breadth of these responses underscores the need for additional research on many species and in many settings.

In line with previous reviews, the effect of biochar in our meta-analysis was generally greater on angiosperms than on gymnosperms, with significant positive effects on growth and

survival response in 43.86% of the angiosperm genera compared with only 25% of the gymnosperm genera included in the 378 studies (Thomas and Gale 2015; Noyce et al. 2017; Fig. 5). Notably, we found the effect size of biochar on Quercus to be negative (-0.07±0.01, N=12). We deliberately chose *Q. rubra* for our field study, because it is a fast-growing and long-lived species of both economic and historic importance to the region, but one whose range is likely to by as much as 50% contract under projected climate change scenarios (Barnes and Wager 2004; Iverson and Prasad 2002). This makes it a suitable candidate for use in active restoration and rehabilitation contexts in the lower peninsula of Michigan, given the likelihood of drier and warmer climates in coming decades. However, it may be the case that Quercus does not typically respond positively to biochar, an idea supported by our experimental findings.

Soil transfers may stimulate the establishment of mycorrhizal networks that can ameliorate some of the water stress on seedlings during establishment by increasing soil-root contact, and biochar can improve soil aggregation and increase soil water retention (Bingham & Simard 2011; Spokas et al. 2012). Thus, in some instances, they may also be used to address water stress in seedlings by increasing water availability. Our results do not support the use of these methods to improve *Q. rubra* growth in similar settings in Michigan, but do suggest that positive effects on seedling survival in the first season may result from such methods. However, the short duration of the field experiment have limited our capacity to assess longer term effects of these treatments. It may also have been the case that the soil volume transferred was not sufficient to successfully establish mycorrhizae. In the future, it would be useful to conduct multi-year studies of the effects of biochar and soil transfer on woody plants in field settings in Michigan. Additionally, detailed assessments of soil nutrient levels, EMF colonization and other measures of soil microbial activity are recommended.

Conclusion

In general, improved knowledge of the effects of different strategies or amendments to restoration sites—as well as plant responses—is needed to guide restoration efforts in the 21st century (Oliet and Jacobs 2012). We foresee that interest in biochar, soil inoculations, and other simple, low-cost ecotechnological interventions will continue to grow, as climate change creates challenging conditions for seedling survival and growth, and as a growing body of literature demonstrates the both the value of restoration and the success of soil amendment techniques in certain climates and species. Our results suggest that biochar and soil transfer may not be an effective way to promote *Q. rubra* seedling establishment in the first year in temperate climates, an idea supported by the results of our meta-analysis. The findings from these field experiments, as well as this systematic review and meta-analysis, will inform future experiments on biochar and soil inoculations and contribute to the body of knowledge on the application of restoration techniques..

Table 1. Mean observed total biomass, shoot:root balance, and mycorrhizal colonization ± SE by site, 2018-2019. Control at UMBS (*) had only one surviving individual.

UMBS - 2018

Treatment	Total biomass (g) ± SE	Shoot:root ratio ± SE	Myco. Colonization ±SE
Control (C) (N = 1)	0.78*	1.11*	na
Biochar (BC) (N = 2)	0.87 ± 0.05	1.25 ± 039	na
Soil Inoculation (SI) (N = 2)	0.88 ± 0.38	1.13 ± 0.64	na
Soil + Biochar (SB) (N = 2)	1.36 ± 0.34	1.16 ± 0.05	na

LTC - 2018

Treatment	Total biomass (g) ± SE	Shoot:root ratio ± SE	Myco. Colonization ±SE
Control (C) (N = 4)	2.25 ± 0.54	0.57 ± 0.34	38.93 ± 5.61
Biochar (BC) (N =9)	1.36 ± 0.32	1.37 ± 0.33	25.83 ± 6.26
Soil Inoculation (SI) (N = 10)	1.55 ± 0.51	0.78 ± 0.22	22.15 ± 5.13
Soil + Biochar (SB) (N = 3)	1.79 ± 0.66	1.31 ± 0.62	18.85 ± 8.64

ARB - 2019

Treatment	Total biomass (g) ± SE	Shoot:root ratio ± SE	Myco. Colonization ±SE
Control (C) (N = 3)	2.12 ± 0.49	1.01 ± 0.15	na
Biochar (BC) (N = 6)	1.35 ± 0.41	1.11 ± 0.21	na
Soil Inoculation (SI) (N = 8)	2.10 ± 0.23	1.35 ± 0.17	na
Soil + Biochar (SB) (N = 5)	1.39 ± 0.15	0.95 ± 0.27	na

Figure 1. Locations of sites within Michigan, USA. UMBS: University of Michigan Biological Station; LTC: Little Traverse Conservancy; ARB: Nichols Arboretum.

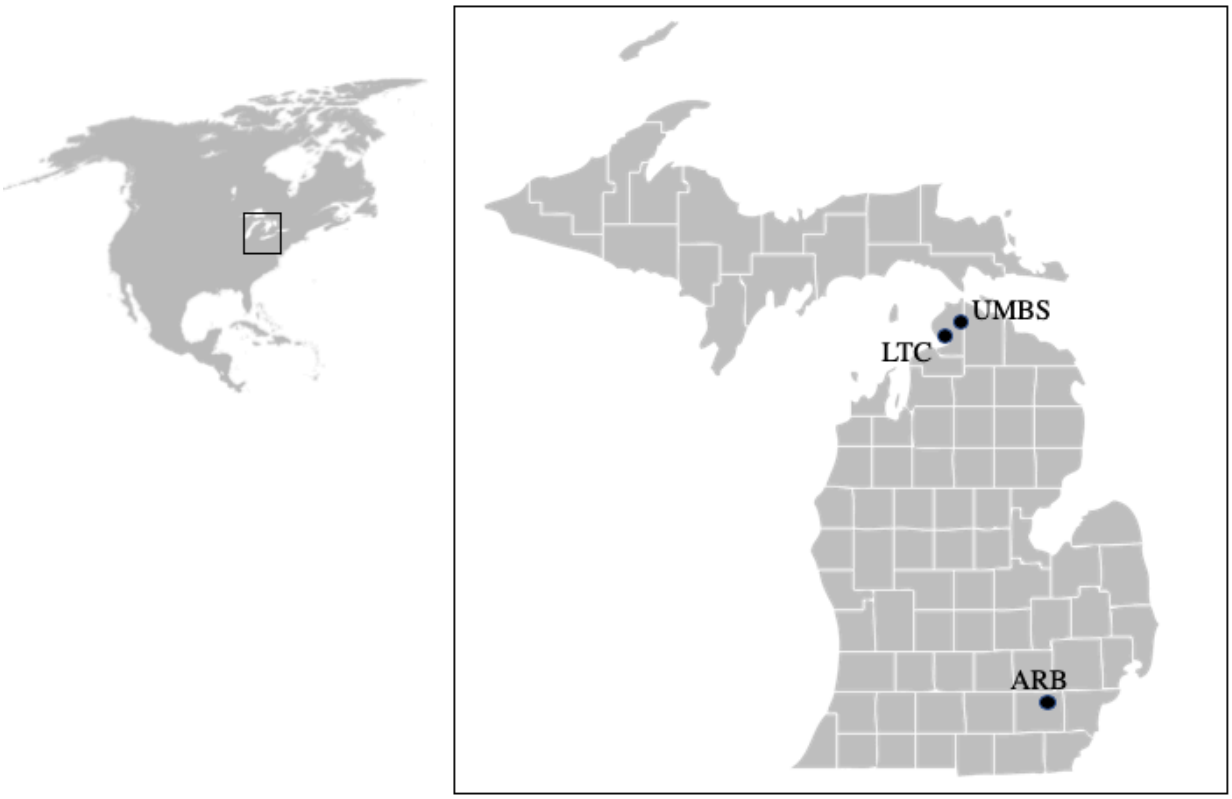


Figure 2. Image of plantings at the Little Traverse Conservancy site (2018).

Figure 3. Image of UMBS site before plantings (2018).

Figure 4. Relative effect size (mean ± 95%CI) of biochar treatments by response variable. Numbers indicated number of observations per category. Confidence intervals that do not include zero are considered statistically significant. Confidence intervals that do not overlap are considered statistically different from each other (indicated by different letters).

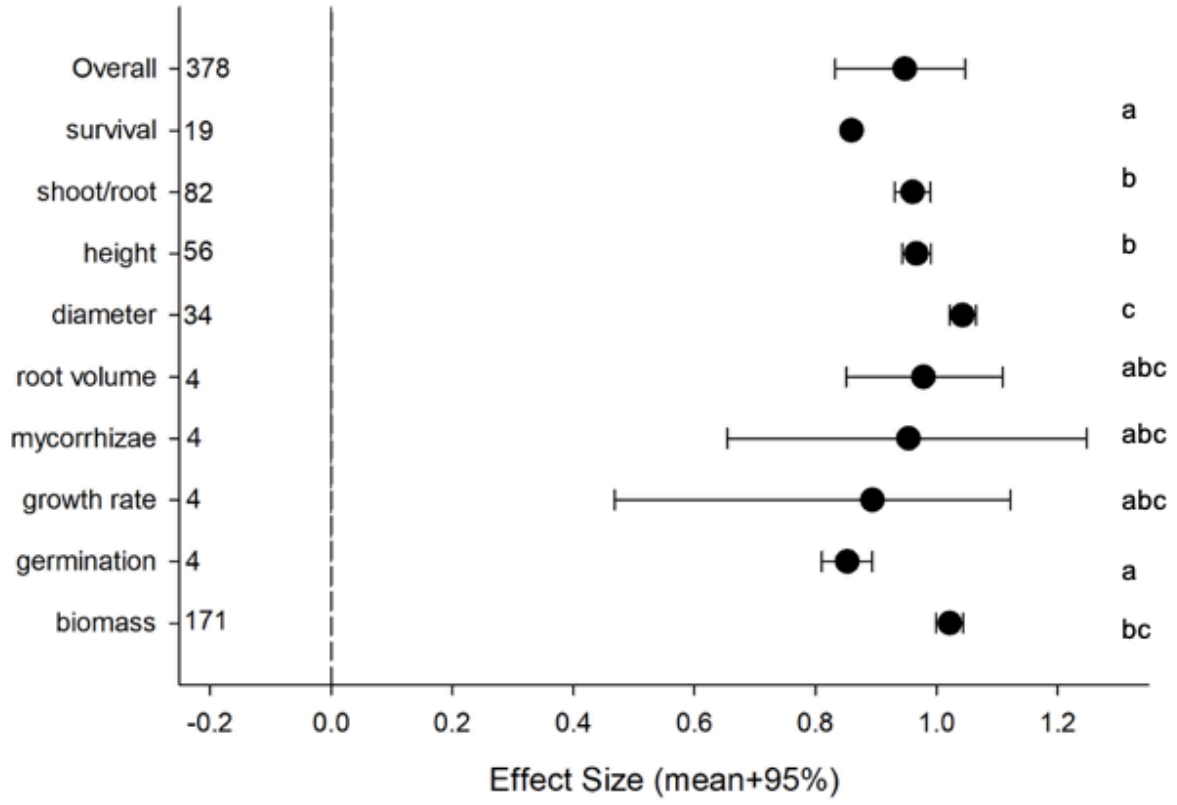


Figure 5. Relative effect size (mean ± 95%CI) of biochar treatments by genus. Numbers indicated number of observations per category. Confidence intervals that do not include zero are considered statistically significant. Shaded areas indicates gymnosperms.

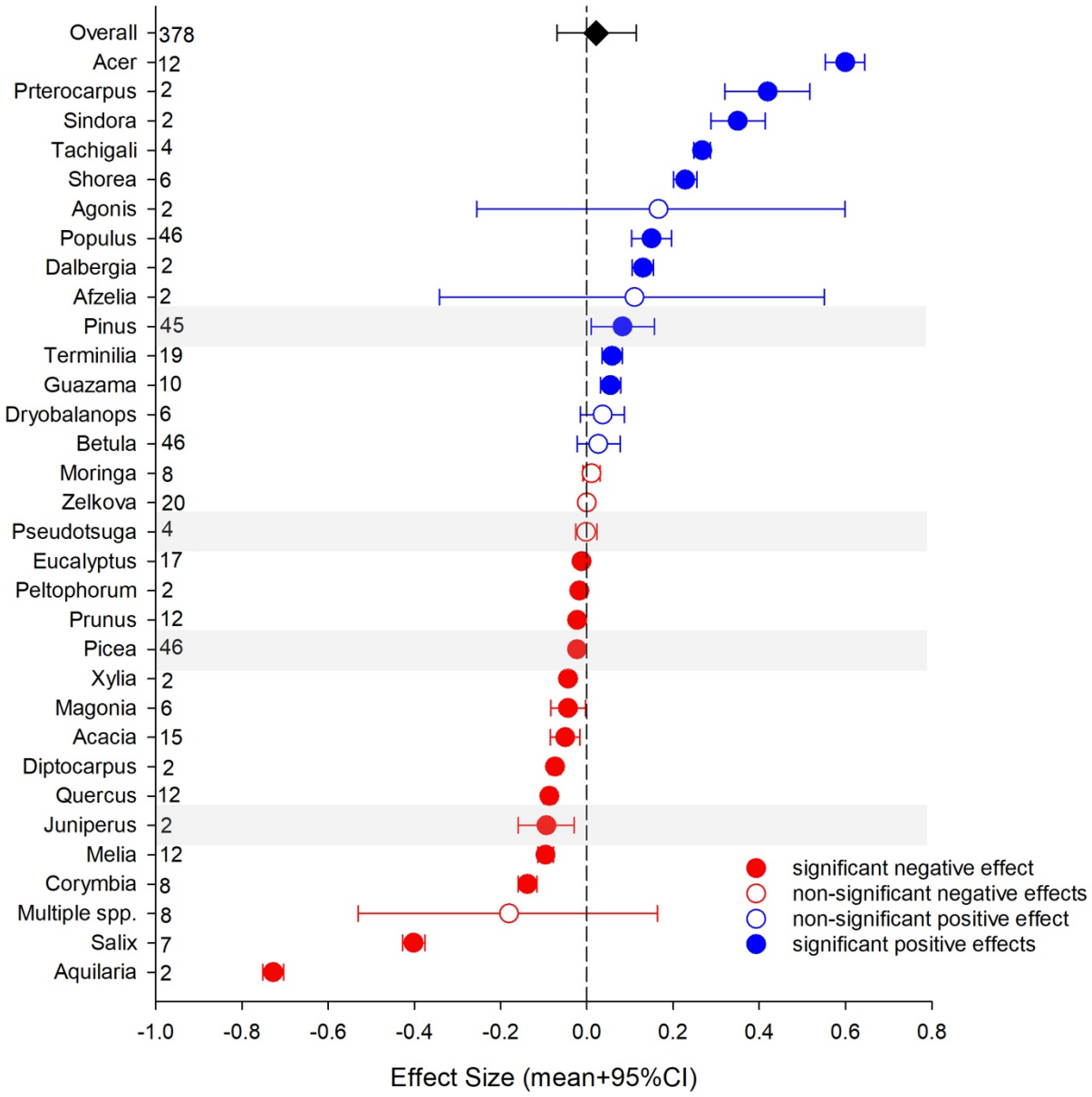


Figure 6. Effect of treatment on survival probability, parameter α. Parameters with 95% CIs that do not overlap are considered statistically different from each other. C: control, BC: biochar, SI: soil inoculation, SB: soil inoculation and biochar.

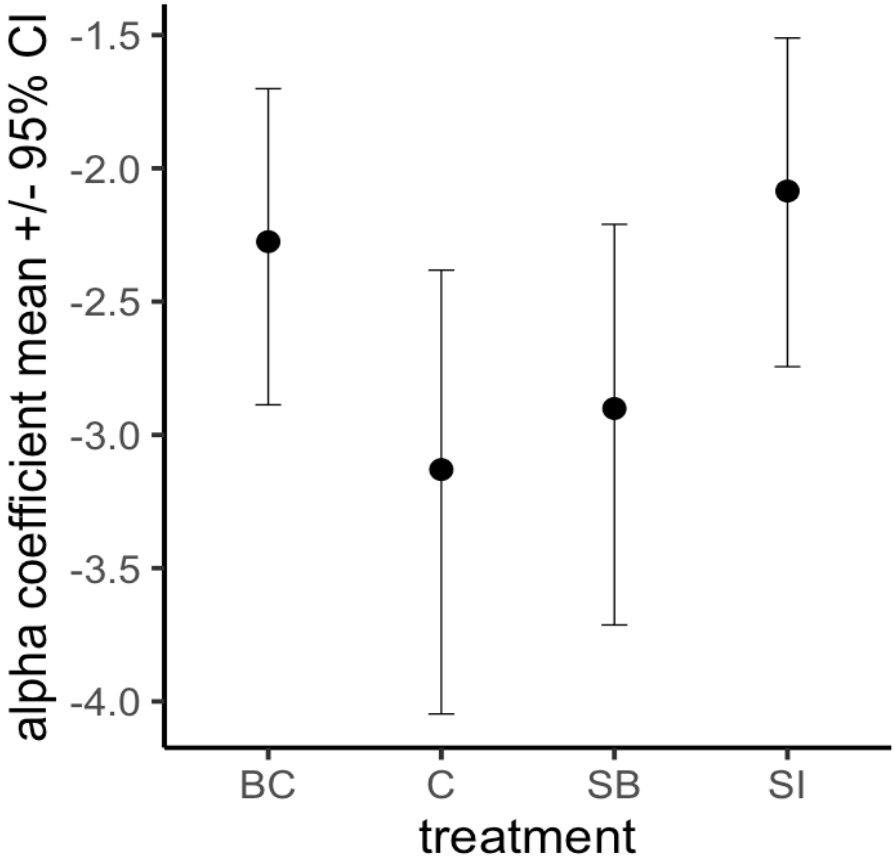
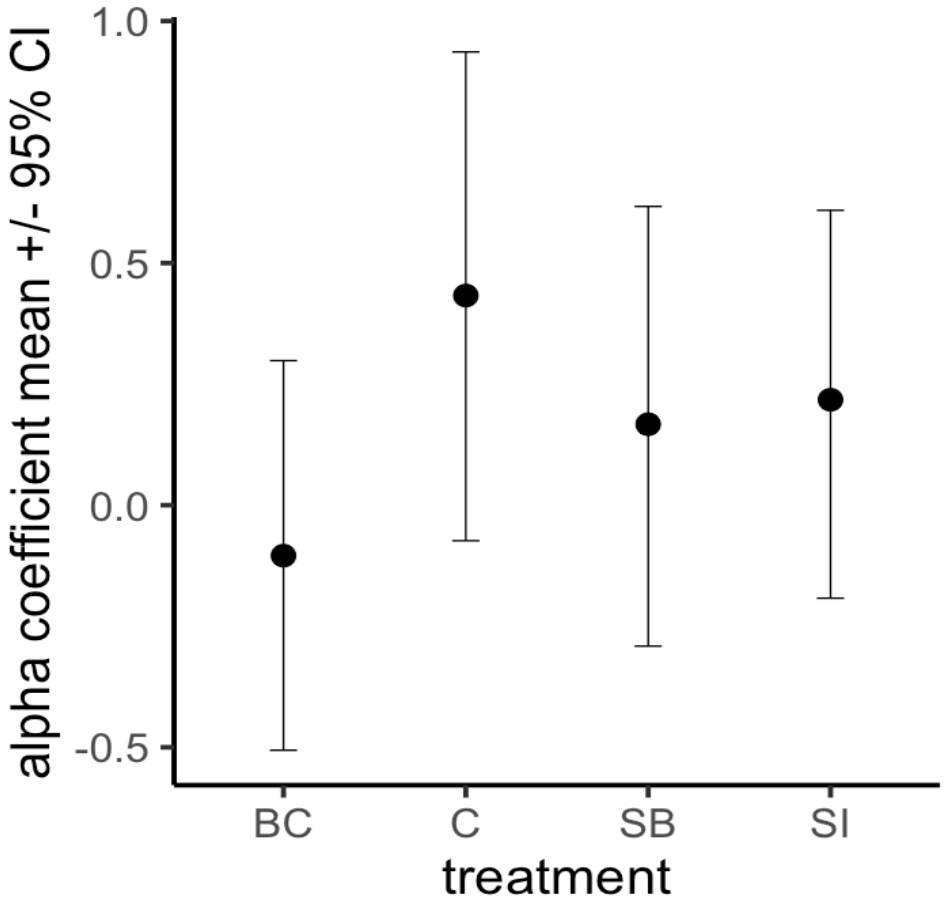


Figure 7. Effect of treatment on biomass, parameter α. C: control, BC: biochar, SI: soil inoculation, SB: soil inoculation and biochar.



Appendix

Appendix A

Table 1A. Search terms for the systematic review.

Database	Search terms
Web of Science	TS=((forest* OR tree* OR seedling* OR "woody plant*") AND (biochar OR charcoal or "black carbon" or "wood ash" or char) AND (restoration OR reclamation OR replanting))
Scopus	TITLE-ABS-KEY((forest* OR tree* OR seedling* OR "woody plant*") AND (biochar OR charcoal OR "black carbon") AND (restoration OR reclamation OR replanting))
Google Scholar	forest* OR tree* OR seedling* OR "woody plant*" AND biochar OR charcoal OR "black carbon" AND restoration OR reclamation OR replanting

Appendix B

Table 1B. Studies included in meta-analysis (N = 26), continued on page 28-29. Format adapted from Thomas and Gale (2015).

Biome	Country	Type	Duration	pH	Feedstock	Temp. (C)	Dosage	Soil transfer	N spp.	Reference
boreal	Canada	field	3 years	7.5	*Populus tremuloides*	350	5 t/ha	no	1	Bieser and Thomas (2019)
tropical	Brazil	field	3 years	5.8	native savanna species	200-500	2.5% v/v, 20% v/v	yes	2	de Farias et al. (2016)
boreal	Canada	pot	< 1 year	unknown	peat	500	10 MT/ha	yes	1	Dietrich and MacKenzie (2018)
boreal	Canada	pot	< 1 year	unknown	peat	500	10 MT/ha	yes	1	Dietrich et al. (2017)
temperate	Australia	field	1.5 years	8.3	*Eucalyptus globulus* and chicken manure	350-500	1 t/ha, 6 t/ha	no	20	Drake et al. (2015)
temperate	Canada	pot, field	< 1 year	8.3	*Picea glauca* wood chips	350-450	5 t/ha, 20 t/ha	yes	1	Kuttner and Thomas (2016)
temperate	United States of America	field	< 1 year	6.7, 7.5	native and invasive grass (n = 2)	350	4.4 t/ha, 3.4 t/ha	no	1	Laungani et al. (2016)
tropical	Peru	nursery	< 1 year	9.906	*Bertholletia excelsa* husks	unknown	1.1 t/ha, 5.5 t/ha	yes	2	Lefebvre et al. (2019)
temperate	Australia	field	3 years	unknown	eucalypt waste	unknown	1 kg/m2	no	2	Macdonald et al. (2017)
arid	Australia	pot	< 1 year	9.22	*Eucalyptus marginata*	700	37 t/ha, 74 t/ha	yes	1	Reverchon et al. (2015)
Mediterranean	Australia	field	< 1 year	unknown	eucalypt wood chips	unknown	20 g per plant	no	2	Ruthrof et al. (2013)

Biome	Country	Type	Duration	pH	Feedstock	Temp. (C)	Dosage	Soil transfer	N spp.	Reference
temperate	Australia	pot	< 1 year	4.9, 6.7	eucalypt hardwoods	500-580	20% v/v	no	2	Somerville et al. (2019)
tropical	Laos	field	4 years	unknown	rice husks	unknown	4 Mg/ha	no	8	Sovu et al. (2012)
boreal	Sweden	pot	< 1 year	6.25-7.42	various tree species (n = 9)	450	2.5 g/pot (3000 kg/ha)	yes	4	Pluchon et al. (2014)
temperate	Republic of Korea	pot	< 1 year	5.1-8.8	pine and oak woodchips, pine cones, crab shells (n = 5)	250	20% v/v	no	1	Cho et al. (2017)
tropical	Nigeria	nursery	< 1 year	8.1	saw dust	~350	5 t/ha, 20 t/ha	yes	1	Fagbenro et al. (2013)
boreal	Finland	pot	< 1 year	unknown	*Picea abies/Pinus sylvestris*; mixed agricultural/forest biomass (n = 2)	unknown	15% v/v, 60% v/v	no	1	Heiskanen et al. (2013)
tropical	Indonesia	pot	< 1 year	8.9	unknown	unknown	5% v/v, 15% v/v	yes	1	Budi and Setyaningsih (2013)
temperate	Australia	pot	< 1 year	7.4	*Acacia pycnantha*	550	5 Mg/ha	no	2	Drake et al. (2016)
temperate	USA	field	2 years	8.4	softwoods	unknown	5 Mg/ha, 20 Mg/ha	no	1	Krapfl et al. (2016)
tropical	Indonesia	nursery	< 1 year	unknown	rice husk	unknown	20% v/v	no	2	Marjenah et al. (2016)
boreal	Canada	pot	< 1 year	7.584	maple saw dust	450	5 t/ha, 50 t/ha	yes	2	Noyce et al. (2017)

Biome	Country	Type	Duration	pH	Feedstock	Temp. (C)	Dosage	Soil transfer	N spp.	Reference
temperate	Republic of Korea	pot	< 1 year	unknown	oak, bamboo (n = 3)	700-1200	40% v/v	no	2	Aung et al. (2018)
temperate	USA	pot	1.5 years	9.18	*Pinus* spp.	550-600	25 Mg/ha	yes	1	Scharenbroch et al. (2013)
tropical	Brazil	pot	< 1 year	6.638	native woody plants	200-500	20% v/v, 30% v/v	no	1	Lima et al. (2015)
temperate	USA	pot	< 1 year	unknown	mixed conifers	980	25% v/v, 50% v/v	no	1	Sarauer and Coleman (2018)

Appendix C

Analysis code. Meta-analysis: Analysis by type of response.

```
#analysis by response
model{

for(i in 1:378){

EStau[i]<-1/(Essd[i]*Essd[i])
ES[i]~dnorm(E[i],EStau[i])

E[i]<-
ESres[response[i]]+SRE[StudyID[i]]

}

for(i in 1:41){SRE[i]~dnorm(0,tau[1])}
for(i in 1:2){
tau[i]<-1/var[i]
var[i]~dunif(0,100)
}
for(sp in
1:9){ESres[sp]~dnorm(R,tau[2])}
R~dnorm(0,0.0001)

}#end
```

Analysis. Meta-analysis: Analysis by genus.

```
model{

for(i in 1:378){

EStau[i]<-1/(Essd[i]*Essd[i])
ES[i]~dnorm(E[i],EStau[i])

E[i]<-ESsp[species[i]]

}

tau<-1/var
var~dunif(0,100)
```

```
 }
 for(sp in 1:32){ESsp[sp]~dnorm(SP,tau)}
 SP~dnorm(0,0.0001)

 }#end

 #initials

 list(var = 1, SP = 0)
```

Analysis. Meta-analysis: Analysis by study.

```
model{

for(i in 1:378){

EStau[i]<-1/(Essd[i]*Essd[i])
ES[i]~dnorm(E[i],EStau[i])

E[i]<-ESS[StudyID[i]]

}

for(i in 1:26){ESS[i]~dnorm(ESm,tau)}

tau<-1/var
var~dunif(0,100)

ESm~dnorm(0,0.0001)

}#end

#initials

list(var = 1, ESm = 0)
```

Appendix D

Table 1D. Seed sources (2018-2019).

Year	Collector	Location
2018	Sheffield Seed Co.	Pennsylvania
2018	Wildtype	Michigan
2019	Wildtype	Michigan

Appendix E

Table 1E. Biochar properties (Wakefield Biochar, 2017).

Attribute	
Pyrolysis temperature	500
Ash (%)	2.22
Moisture (%)	54.44
pH	7.4

Elemental composition	
Bulk density (g/cm -3)	0.48
Total Carbon	40
Nitrogen (% wt)	0.27
Total Phosphate (mg/kg)	2.06
Potassium (mg/kg)	280
Sulfur (% wt)	0.014
Hydrogen	0.18
Oxygen (% wt)	2.77
Calcium (mg/kg)	1881
Copper (mg/kg)	2.45
Iron (mg/kg)	271
Magnesium (mg/kg)	558
Manganese (mg/kg)	107
Zinc	2.09
Particle Size <0.5 mm (%)	22.4
Particle Size <1 mm (%)	70.1
Particle Size <2 mm (%)	93.9

Appendix F

Model code for OpenBugs 3.2.3.

Survival (2018-2019)

```
model{

for(i in 1:648){

sur[i]~dbern(p[i])
p[i]<-max(0,p0[i])

logit(p0[i])<-A[treat[i]]+beta*PheightS[i]+PRE[plot[i]] #planted height standardized

}

#prior
for(i in 1:4){A[i]~dnorm(0,0.0001)

survp[i]<-exp(A[i])/(1+exp(A[i])) #predicted survival at average planted height

}
beta~dnorm(0,0.0001)
for(i in 1:9){PRE[i]~dnorm(0,tau)}

tau~dgamma(0.001,0.001)
var<-1/tau


} #end model

#initials
list( tau =1, A =c(0,0,0,0), beta = 0    )
```

Biomass (2018-2019)

```
model{

for(i in 1:55){

biomass[i]~dlnorm(B[i],tau[1])
b.h[i]~dlnorm(B[i],tau[1])      #predictions

B[i]<-alpha[i]+beta*Pheights[i]+SRE[site[i]]
```

```
alpha[i]~dnorm(A[treat[i]],0.0001)
}

#priors
for(i in 1:2){
tau[i]~dgamma(0.001,0.001)
var[i]<-1/tau[i]
}

for(i in 1:4){A[i]~dnorm(0,0.0001)}

for(i in 1:3){SRE[i]~dnorm(0,tau[2])}

beta~dnorm(0,0.0001)

} #end model

#initials
list(tau = c(1,1), A = c(1,1,1,1), beta = 0 )
```

Mycorrhizal fungi colonization (2018)

```
model{

for(i in 1:26){

#myco ECTO –
ECTO[i]~dpois(ECTOm[i])
ECTO.h[i]~dpois(ECTOm[i]) #predictions

ECTOm[i]<-alpha[treat[i]]+beta*PlantHeightS[i]+SRE[site[i]]

}

beta~dnorm(0,0.0001)
for(t in 1:4){ #treatments
alpha[t]~dlnorm(1,0.001)
}

for(i in 1:3){# sites
SRE1~dnorm(0,tau)
}
tau~dgamma(0.001,0.001)
var<-1/tau
}
```

Appendix G

Table 1G. Survival Model Parameters, posterior mean SD and 95%CI. C: control, BC: biochar, SI: soil inoculation, SB: soil inoculation and biochar.

	Mean	St. Dev.	95% CI	
αC	-3.13	0.426	-4.047	-2.382
αBC	-2.275	0.2986	-2.887	-1.701
αSI	-2.085	0.3091	-2.744	-1.511
αSB	-2.901	0.3916	-3.713	-2.21
β initial plant height	0.2243	0.1493	-0.06934	0.5216
σ^2var	0.2776	0.428	0.001197	1.353

Table 2G. Biomass model parameters, posterior mean SD and 95%CI. C: control, BC: biochar, SI: soil inoculation, SB: soil inoculation and biochar.

	Mean	St. Dev.	95% CI	
αC	0.433	0.2531	-0.07337	0.9361
αBC	-0.1042	0.2008	-0.5061	0.2986
αSI	0.2178	0.1966	-0.1921	0.609
αSB	0.1672	0.2273	-0.2911	0.617
β initial plant height	0.1267	0.2194	-0.3096	0.5459
σ^2var [1]	0.1927	0.007786	0.001224	0.4655
σ^2var [2]	0.1663	0.007917	0.001137	0.4572
σ^2var [3]	0.08907	0.006511	7.09E-04	0.5613

Table 3G. Mycorrhizal colonization model parameters, posterior mean SD and 95%CI. C: control, BC: biochar, SI: soil inoculation, SB: soil inoculation and biochar. Colonization recorded from LTC site, 2018 only.

	Mean	St. Dev.	95% CI	
αC	38.81	3.122	32.86	45.18
αBC	25.81	1.991	22.05	29.74
αSI	24.35	1.771	20.96	27.93
αSB	18.69	2.511	14.08	23.98
β initial plant height	0.1267	0.2194	-0.3096	0.5459

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