

Article

Effects of Biochar on Drought Tolerance of *Pinus banksiana* Seedlings

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Abstract: Drought is a major stressor of tree seedlings regarding both natural and artificial regeneration, especially in excessively drained, sandy outwash soils. While climate change is expected to cause an increase in the total annual precipitation in the Upper Midwest, USA, the timing of the precipitation is predicted to result in longer periods of drought during the growing season. Biochar, a material created through the pyrolysis of organic matter, such as wood waste, has been proposed as a soil amendment that may increase the water holding capacity of a soil. Biochar has mostly been studied in agricultural settings, and less is known about the impact of biochar on forest soils and tree seedlings. We used a greenhouse experiment to test the ability of biochar to improve the drought tolerance of jack pine (*Pinus banksiana*) seedlings via increased soil water holding capacity. The seedlings were planted in sandy soil treated with three levels of biochar (none, 3% by weight, and 6% by weight) in two experiments, one manipulating the timing of drought onset and the other controlling the amount of water that seedlings received. Our results showed no significant effects of biochar on seedling survival, growth, or physiology under drought conditions. While this outcome did not support the hypothesis that biochar would increase seedling performance, the biochar amendments did not negatively affect seedlings, indicating that biochar may be added to soil for carbon storage without having negative short-term impacts on tree seedlings.



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1. Introduction

Climate change will have a strong effect on the timing, frequency, and amount of precipitation globally. While some regions are expected to receive decreased total precipitation, other regions, including the Upper Midwest, USA (defined as the states of Michigan, Minnesota, and Wisconsin), are expected to experience an increase in annual precipitation in the form of fewer, more intense events with longer periods of drought between them [1]. This increased precipitation intensity is expected to lead to more runoff and decreased annual mean soil moisture [2]. Decreasing soil moisture and increasing periods of drought can result in increased stress for plants, especially in the excessively drained, sandy glacial outwash soils of the region [3]. Vulnerability to this increased drought stress will likely depend on drought severity, timing of drought (early growing season, late growing season, dormant season), and individual plants. Young trees, especially newly planted tree seedlings, are likely to be particularly vulnerable [3]. Tree seedlings have smaller root systems and fewer carbohydrate reserves than mature trees, contributing to their lower tolerance to drought stress [4]. Additionally, newly planted seedlings are not connected to mature trees in the stand via mycorrhizal networks, which have been shown to decrease seedling drought stress [5]. These facts are particularly concerning since forest management practices that include tree planting (afforestation, reforestation, assisted migration, etc.)

are increasingly being implemented to meet goals of increasing carbon storage and/or increasing resilience to climate change [6].

In response to the increased threat of drought stress for seedlings, forest and natural resource managers are interested in exploring treatments that could increase soil water holding capacity. One potential option is biochar, which is a product created through pyrolysis of wood and wood waste [7,8]. However, the results of studies analyzing the effects of biochar on water repellency, water infiltration, and soil drying have been mixed. Many factors likely contribute to variable results, including differences in biochar feedstock, particle size distribution, freshness, application method, soil type, and time since application [7,9–12]. Most research related to the effects of biochar on plant growth has been performed in agricultural settings, but there has been growing interest and new research on the effects of biochar on tree seedling growth and survival [13].

The results of previous studies of biochar additions in forested settings have shown positive, neutral, and negative effects. In field settings, several studies have shown positive effects of biochar on the growth of *Pinus* species, including *Pinus sylvestris* L. in Sweden [14] and Finland [15] and *Pinus resinosa* Aiton in Ontario, Canada [16]. However, two separate field studies in Minnesota, USA, did not find a strong effect of biochar on the growth or survival of jack pine (*Pinus banksiana* Lamb.) seedlings in sandy soils [17,18]. A Wisconsin, USA, field study found no significant effect of biochar on the height or diameter growth of *P. resinosa* seedlings [8], and field studies with other conifer species have also found that biochar did not affect seedling growth [19,20]. Even in controlled settings in greenhouses or growth chambers, the effects of biochar on tree seedling growth have not been consistent across studies. In greenhouse experiments, biochar was found to increase the shoot biomass of *Pinus contorta* (Dougl. Ex Loud.) var. *latifolia* (Engelm.) [21], increase the height (but not biomass) of *Pinus ponderosa* (Lawson & C. Lawson) seedlings [22], and increase the growth and quality of *Eucalyptus grandis* L. seedlings [23]. On the other hand, experiments in controlled settings found no strong effects of biochar on *Populus euramericana* Guinier seedling growth [24], no effect of biochar on the height or root volume of *Picea abies* L. Karst. seedlings [25], and reduced growth of *Pseudotsuga menziesii* (Mirb.) Franco when biochar was added to a peat growth medium [26].

While studies of the effects of biochar on seedling growth have had varied results, most investigations into the effects of biochar on plant drought tolerance have shown biochar to have positive effects. In agricultural settings, biochar application has been shown to mitigate drought stress in tomato plants, soybeans, cabbage, and many other species [27–31], but Hansen et al. found that woody gasification biochar did not affect growth of barley under drought conditions [32], and Mannan et al. found that, while the addition of biochar increased soybean biomass under drought conditions, it did not increase plant water uptake [33]. Fewer studies have examined the impacts of biochar on drought stress in trees, but most have still found positive impacts. In *Pinus* species, biochar helped *Pinus thunbergii* Parl. seedlings to maintain xylem water potential longer after drought in sandy soil [34], and *P. ponderosa* seedlings grown in a greenhouse with soilless media required less irrigation when more biochar was added to the growth media [35]. In hardwood trees, biochar has been found to have some protective effect from drought stress for *Pyrus ussuriensis* Maxim [36], and *Tilia × europaea* L. seedlings in sandy loam soil were better able to tolerate and recover from drought when biochar had been integrated into the soil [37]. Two studies of different *Quercus* species have found interesting results; in one study with *Quercus castaneifolia* C.A.M., at least 3% biochar by weight added to the soil was required to see positive impacts on photosynthesis, conductance, and transpiration under the driest conditions [38], but a second study of *Quercus brantii* Lindl. found that addition of smaller amounts of biochar (1% or 2% by weight) increased seedling growth and response under dry conditions, while 3% biochar had a negative effect [39]. An additional study that included another *Quercus* species (*Quercus ilicifolia* Wangerh.) found that, in dry conditions, photosynthesis and stomatal conductance were lower in pots with oak biochar compared to the controls [40].

In forested settings, there are additional constraints that influence the feasibility of using biochar as a soil additive. Unlike agricultural fields, which may have farming equipment on site multiple times during the growing season and can be tilled annually, forested stands can often go decades between treatments, occur on uneven ground, occur in high slope areas (>50% slope), and contain multiple age classes of trees and important understory vegetation [41]. Due to these constraints, forest researchers and managers are exploring locations where biochar may be most impactful. Within the Upper Midwest, one location where biochar has been hypothesized to be most impactful is in excessively drained, sandy outwash soils, which are predicted to be disproportionately impacted by climate change [42]. These soils generally have low water holding capacity, arising from the particle size distribution and low amounts of soil organic matter [43]. While drought-tolerant species are common on these soil types, historically frequent, low-intensity rainfall events during the growing season likely reduced drought stress. Predicted shifts in the timing and intensity of precipitation will likely increase drought stress and make regeneration and planting success in these soil types more unpredictable [44]. Within this region and on these soil types, jack pine is commonly used by natural resource management organizations (US Forest Service and State Departments of Natural Resources (DNR)) in restoration through natural and artificial regeneration methods due to the species' tolerance of drought and sandy, nutrient-poor soils [45].

Given the limited initial effects of biochar on the survival and growth of seedlings in the field experiments described above (especially those in sandy soils [8,17,18]), there is a need for more controlled studies to robustly assess the potential drought-protective effects of biochar on conifer tree seedlings in sandy, outwash soils. Natural resource managers lack clear information about the potential trades-offs related to incorporating biochar into forest management operations in the Upper Midwest. To address this information gap, we designed a greenhouse study in which two experiments were implemented to test different potential ways in which biochar additions could influence the survival of jack pine seedlings, as well as mimic potential climate change projections related to precipitation in the region. We aimed to answer the following questions with our two experiments, in sandy, well-drained soil: (1) Does the addition of biochar affect the survival of jack pine seedlings during prolonged drought at different points in the growing season? and (2) Does the addition of biochar to soil affect the growth or physiology of jack pine seedlings under low water conditions? For growth responses, we chose to focus on seedling height and diameter (as opposed to seedling or root biomass) because these measurements are used by foresters and practitioners and can be readily measured in field settings.

2. Materials and Methods

This study was conducted from May to October 2021 in a greenhouse on the University of Minnesota campus in St. Paul, Minnesota, USA. The air temperature of the greenhouse was recorded every 15 min for the duration of the experiment using five HOBO U23-002 temperature/RH sensors (Onset) placed across the benches used for the experiments. The mean greenhouse air temperature throughout the experiment was 22.6 °C, the maximum air temperature was 33.9 °C, and the minimum air temperature was 11.3 °C. The mean daily maximum air temperature was 27.2 °C, and the mean daily minimum air temperature was 19.2 °C. No additional lighting was used in the greenhouse, only ambient sunlight. The greenhouse was whitewashed on 3 June 2021 (week 3 of the experiment), which generally prevented the maximum daily temperature in the greenhouse from exceeding the outdoor maximum daily temperature.

The study design consisted of two experiments: a drought onset experiment and a water amount experiment (see details below).

2.1. Drought Onset Experiment

The drought onset experiment was a 3 × 3 factorial design with three times (treatments) when simulated drought was initiated to reflect early season, mid-season, and

later season droughts, crossed with three amounts of biochar added to soil. For each treatment, there were 15 replicates (pots). One jack pine seedling was planted in each pot (12.7 cm × 12.7 cm × 30.5 cm) on 17 May 2021 (week 1 of the experiment). The jack pine seedlings were 1-year-old, container grown seedlings, grown in Brighton, Michigan, USA, from seed sourced from Douglas, County, Wisconsin, USA. The mean seedling basal diameter was 2.58 mm (± 0.03 mm SE), and the mean height to terminal bud was 17.4 cm (± 0.2 cm) at the time of planting. There were no significant differences for initial seedling height or diameter by group prior to treatments. Pots were randomly placed on two benches within the greenhouse on a 10 cm × 15 cm grid spacing.

2.1.1. Biochar

The biochar used in this experiment was produced by the University of Minnesota Natural Resources Research Institute (NRRI) from black ash (*Fraxinus nigra* Marsh.), which underwent pyrolysis at 550 °C in a Heyl & Patterson Rotary Calciner Kiln. The biochar properties are shown in Table 1.

Table 1. Properties of black ash biochar used in study.

Property	Value
C (w%)	84.04
H (w%)	2.47
N (w%)	0.48
O (w%)	7.18
Ash (w%)	5.83
VM (w%)	15.06
Fixed C (w%)	79.12
H:C molar ratio	0.35
O:C molar ratio	0.064
pH	7.36
Conductivity (S/m)	432
Liming (%CCE)	3.5
CEC (cmol cations per kg char)	1.6

The biochar treatments consisted of three amounts of biochar combined by mixing completely with a 95% sand/5% garden soil mixture: 0% biochar by weight, 3% biochar by weight, and 6% biochar by weight (Table S1). This mixture was chosen to mimic the sandy soils in which jack pine commonly grows in the Upper Midwest region, as well as limit the water holding capacity of the soil, and to maximize drought stress. The selected biochar application rates were similar to those in other studies that found biochar additions to increase soil water holding capacity or seedling drought tolerance [7,38]. The total mass of the mixture was adjusted for each treatment combination to achieve approximately equal volumes in each pot. The sand used in this experiment was standard play sand purchased from a hardware store. The garden soil was MiracleGro All Purpose Garden Soil. In each pot, 48 g of Osmocote Plus 15-9-12, 8- to 9-month fertilizer was mixed into the top 20 cm of soil before seedlings were planted to ensure that nutrients were not limiting to seedling growth during the experiment. The fertilizer added 7.2 g of total nitrogen, 4.3 g of phosphate, and 5.8 g of potash plus micronutrients to each pot (see product label for details on micronutrients).

2.1.2. Water Treatment

All seedlings (all treatments) were watered to greater than field capacity for the first 2 weeks. On the first day of the experiment (planting day), all seedlings received 1 L of water. For the remaining days of weeks 1 and 2, seedlings received 500 mL of water every other day. Tap water was used in all instances.

The three water treatments in the drought onset experiment were defined by the timing of drought onset, with drought conditions beginning in week 3 (W3, early season onset),

week 7 (W7, mid-season onset), or week 11 (W11, later season onset). For each treatment, seedlings received 200 mL of water every other day until the first day of their drought onset week (W3, W7, or W11). Once the drought period began, water was completely withheld from the seedlings to simulate extreme drought conditions until the end of the experiment after 21 weeks.

2.2. Water Amount Experiment

The water amount experiment was a 2×3 factorial design with two water amounts (high vs. low water availability) and three amounts of biochar added to the soil. For each treatment, there were 15 replicates (pots). The planting conditions, seedling source, and seedling size were as described for the drought onset experiment in Section 2.1. Pots were randomly placed on two benches within the greenhouse at a spacing of 10 cm $\times$ 15 cm between pots.

2.2.1. Biochar

The biochar treatments (0% biochar by weight, 3% biochar by weight, and 6% biochar by weight) and biochar source and characteristics were the same as described for the drought onset experiment (Section 2.1.1, Table 1 and Table S1).

2.2.2. Water Amount Treatment

All seedlings (all treatments) were watered to greater than field capacity for the first two weeks (establishment period) as described for the drought onset experiment (Section 2.1.2). The two water treatments in the water amount experiment were defined as low and high. The low water treatment (20 mL every other day) represented the average monthly rainfall for the driest month of summer 2020 in Cloquet, Minnesota, USA (June 2020, 0.07 cm/day) [46]. The high water treatment (200 mL every other day) represented 10 times the low water treatment. Water level treatments began in week 3 and continued to the end of the experiment.

2.3. Data Collection

For both experiments, a qualitative assessment of the condition of each seedling was carried out every other day beginning in week 3 until the seedling was determined to be dead. Seedlings were given a condition score of 2 (seedling healthy, green needles, no wilting), 1 (seedling in poor health, greater than 30% of needles turned brown and/or seedling wilted), or 0 (dead, all needles brown and dry) (Figure 1). The day of seedling mortality was defined as the first day on which a seedling received a condition rating of 0 that was followed by another 0 condition rating 2 days later. For example, if a seedling received a condition rating of 0 on 27 July and 29 July, the day of seedling death would be 27 July. If a seedling received a condition rating of 0 on 27 July but then received a condition rating of 1 on 29 July (possible because the condition ratings were qualitative visual assessments only), that seedling would not yet be considered dead. Additionally, individual photographs were taken of all living seedlings in week 2, week 3, and every other week thereafter to track health status (Figure 1).

Height and basal diameter measurements were collected for seedlings in both experiments at the time of planting (week 1), after the establishment period (week 3), and then every 4 weeks thereafter (weeks 7, 11, 15, 19). These measurements coincided with the start of drought treatments in the drought onset experiment. Final height and diameter measurements were taken when seedlings were determined to be dead or at the end of the experiment (week 21).

At the time of planting, a ring of paint was applied to the stem of each seedling just above the root collar, and the basal diameter was measured at this location each time. Using digital calipers, two perpendicular measurements of basal diameter were taken to the nearest 0.1 mm and averaged to determine the mean diameter. Heights of seedlings

were measured from the soil surface to the terminal bud (not including needles) to the nearest 0.1 cm.

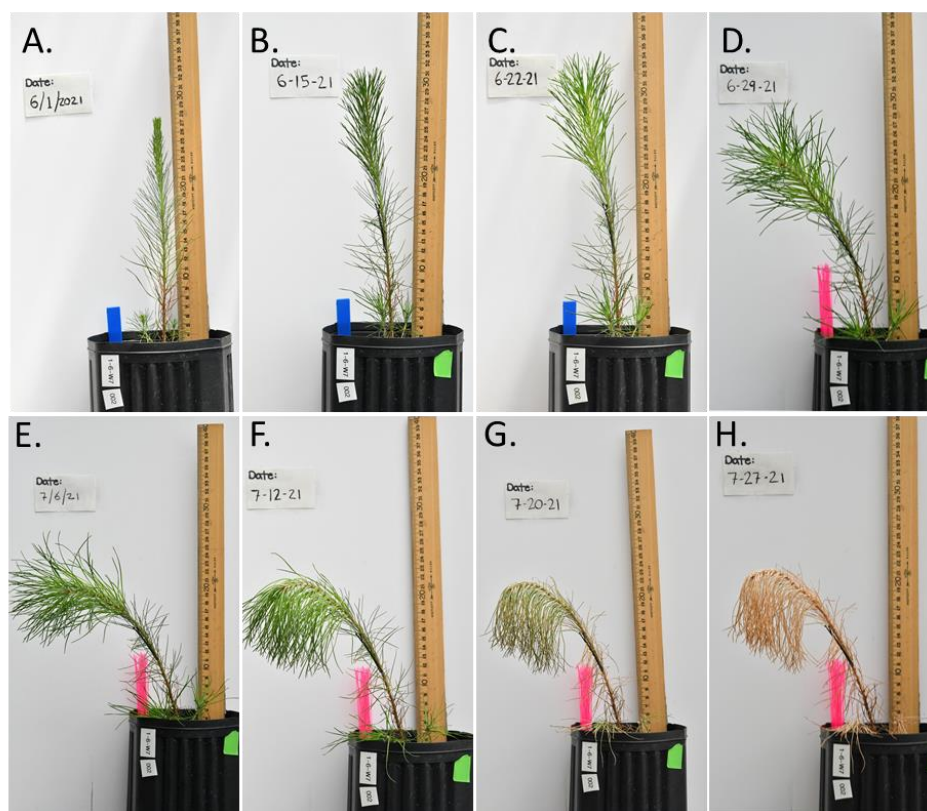


Figure 1. Time series photos of seedling 002. Seedling 002 was from the drought onset experiment and received the 6% biochar treatment with drought onset in week 7 (28 June, beginning with photo (D)). This seedling was rated as healthy (health status = 2) in photos (A–E), unhealthy (health status = 1) in photos (F,G), and dead (health status = 0) in photo (H).

In the water amount experiment, five of the 15 replicates for each treatment combination were selected for physiology measurements. For these seedlings, light-saturated photosynthesis, transpiration, and stomatal conductance were measured using an LI-6400 (LI-COR Biosciences). These measurements were taken for live seedlings every other week in weeks 3–15 and then weekly in weeks 16–21. For each seedling, a group of needles were clamped together using a bobby pin and spread out to minimize overlap so that the same needles could be measured each week. Measurements were taken between 8:30 am and 4:30 pm local time. All measurements for each week occurred on the same day. Environmental conditions within the LI-6400 leaf chamber were set as follows: CO₂ concentration of 400 $\mu\text{mol}\cdot\text{mol}^{-1}$, block temperature of 24 °C, PAR of 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and relative humidity of 45–65%. For each seedling, once the readings had stabilized, three readings were taken approximately 10 s apart. At the end of the experiment or after the seedling had died, the needles within the bobby pin used for LI-6400 readings were removed from the tree, clipped to the area of the LI-6400 chamber, and scanned, and the projected area was measured using ImageJ software [47].

2.4. Data Analysis

In the drought onset experiment, total days of seedling survival were calculated as the number of calendar days from the start of drought treatment (W3: 5/31/2021, W7: 6/28/2021, W11: 7/26/2021) to the day the seedling was noted as dead (condition score = 0, see above). Five seedlings in the W11 treatment were dead or in poor health (condition

rating = 0 or 1) before the start of the drought treatment; these seedlings were dropped from the survival analysis. Analysis of variance (ANOVA, type III tests) was used to determine the effects of biochar and water treatments on the number of days of seedling survival.

In the water amount experiment, seedling diameter growth was calculated as the change in mean basal diameter from planting to seedling death or the end of the experiment (week 21). Additionally, in this experiment, seedling height growth was calculated as the change in bud height from planting to seedling death or the end of the experiment (week 21). Mixed model analysis of covariance (ANCOVA) was used to determine effects of biochar and water treatments on diameter and height growth. Initial measurements of seedling diameter or height were used as covariates.

All statistical analyses were performed using R statistical software [48]. ANOVAs and ANCOVAs used the nlme R package [49]. If significant main effects were found, Tukey's HSD was used to determine pairwise differences between treatments; $p < 0.05$ was considered significant. For analysis of change in seedling diameter in the water amount experiment, assumptions of constant variance were not met and the dependent variable (Δ Diameter) was transformed using a square root transformation: $\sqrt{\Delta\text{Diameter} + 1}$. For analysis of height growth in the water amount experiment, two observations were considered to be outliers because the absolute value of the observation's standardized residual was greater than 3. These observations were for seedlings 225 (low water, 0% biochar) and 285 (high water, 0% biochar); these seedlings were removed from analysis.

Data for photosynthetic rate, stomatal transpiration rate, and stomatal conductance rate obtained from the LI-6400 were analyzed using repeated measures ANOVA with the nlme R package and an autoregressive level (1) (AR [1]) covariance matrix [49]. Models for physiology measurements were assessed using data from week 3 (the first measurements in the experiment) through week 15. Beginning in week 16, in the low water treatment, only two of the original five replicates remained alive for both the 3% and the 6% biochar treatments, so measurements from these additional weeks were not used.

3. Results

3.1. Drought Onset Experiment

The mean number of days the seedlings survived after drought onset was 49.5 days; minimum survival was 21 days, and the longest-surviving seedling died after 109 days. Neither biochar nor drought onset treatment had a significant effect on seedling survival ($p = 0.6828$ and $p = 0.3205$, respectively). The interaction between biochar and drought onset was also not significant ($p = 0.1501$). Although the effect was not significant, seedlings that experienced drought onset in week 7 and week 11 seemed to survive slightly longer (mean survival of 53 and 54 days, respectively) than seedlings that experienced drought onset in week 3 (mean survival = 41 days, Figure 2).

3.2. Water Amount Experiment

3.2.1. Height and Diameter Growth

Seedlings grew significantly more in diameter and height under the high water treatment compared to the low water treatment (Table 2). The mean change in seedling basal diameter from planting to the end of the experiment was $0.34 (\pm 0.15)$ mm for seedlings in the low water treatment and $3.44 (\pm 0.33)$ mm for seedlings in the high water treatment (Figure 3a). The mean change in seedling height from planting to the end of the experiment was $9.8 (\pm 0.3)$ cm for seedlings in the low water treatment and $12.0 (\pm 0.4)$ cm for seedlings in the high water treatment (Figure 3b). The effect of biochar treatment on seedling diameter growth was not significant, but the effect of biochar treatment on height growth was nearing significance at $p = 0.076$ (Table 2). Pairwise comparisons of biochar treatments using Tukey's HSD did not indicate differences even at the $p < 0.1$ level, but seedlings receiving the 6% biochar treatment may have trended slightly taller than seedlings receiving the 0% and 3% biochar treatments ($p = 0.192$ and $p = 0.154$, respectively, Figure 3b). Interactions between biochar treatment and water treatment were not significant (Table 2).

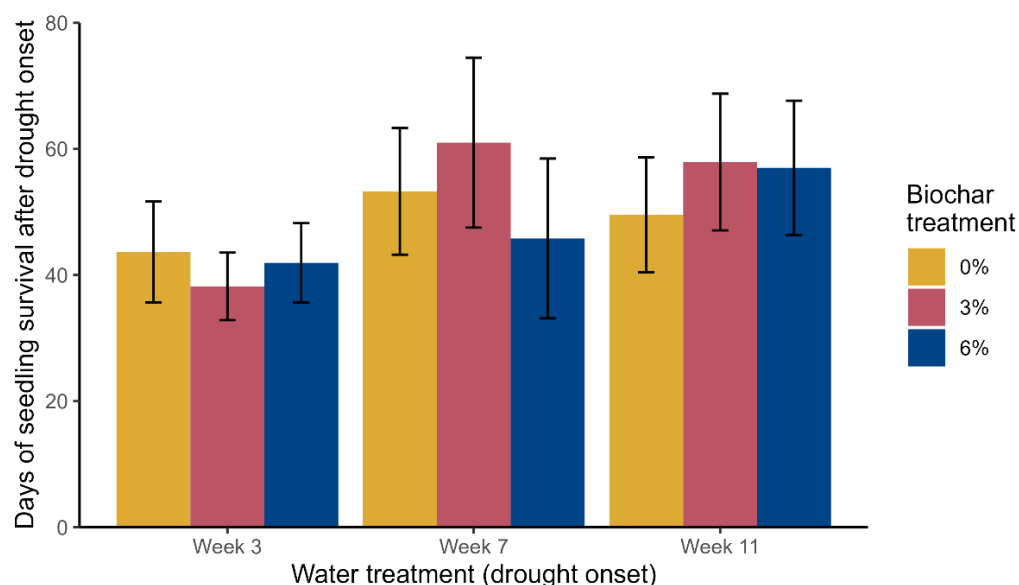


Figure 2. Mean seedling survival by biochar and drought onset treatment. Neither timing of drought onset nor biochar treatment had a significant effect on seedling survival. Error bars indicate 95% confidence intervals; $n = 15$ for all treatment combinations except week 11, 3% biochar ($n = 13$) and week 11, 6% biochar ($n = 12$).

Table 2. F-statistic probabilities for seedling growth models (Change in measurement ~ Initial measurement + Biochar * Water). Asterisks (*) indicate interactions between terms. Significant p -values ($p < 0.05$) are shown in bold.

Term	Df	Change in Diameter (mm)	Change in Height (cm)
		p -Value	p -Value
Covariate (initial measurement)	1	0.9	0.002
Biochar	2	0.515	0.076
Water	1	<0.001	<0.001
Biochar * Water	2	0.641	0.503

3.2.2. Physiology Measurements

Models for physiology measurements were assessed using data from week 3 (the first measurements in the experiment) through week 15.

Biochar treatment did not have a significant effect on any of the three physiology measurements collected (mean photosynthetic rate, mean stomatal transpiration rate, and mean stomatal conductance rate). For each of the physiology measurements, week and water treatment were the only significant model parameters (Table 3). Photosynthetic rate, stomatal transpiration rate, and stomatal conductance rate each generally decreased weekly throughout the experiment for both water treatments. Seedlings receiving the high water treatment had higher photosynthetic, transpiration, and conductance rates than those receiving the low water treatment throughout the experiment (Figure 4). Beginning in week 9, rates of stomatal transpiration and conductance were near zero for seedlings receiving the low water treatment, especially for seedlings with a health rating of “1” (poor health).

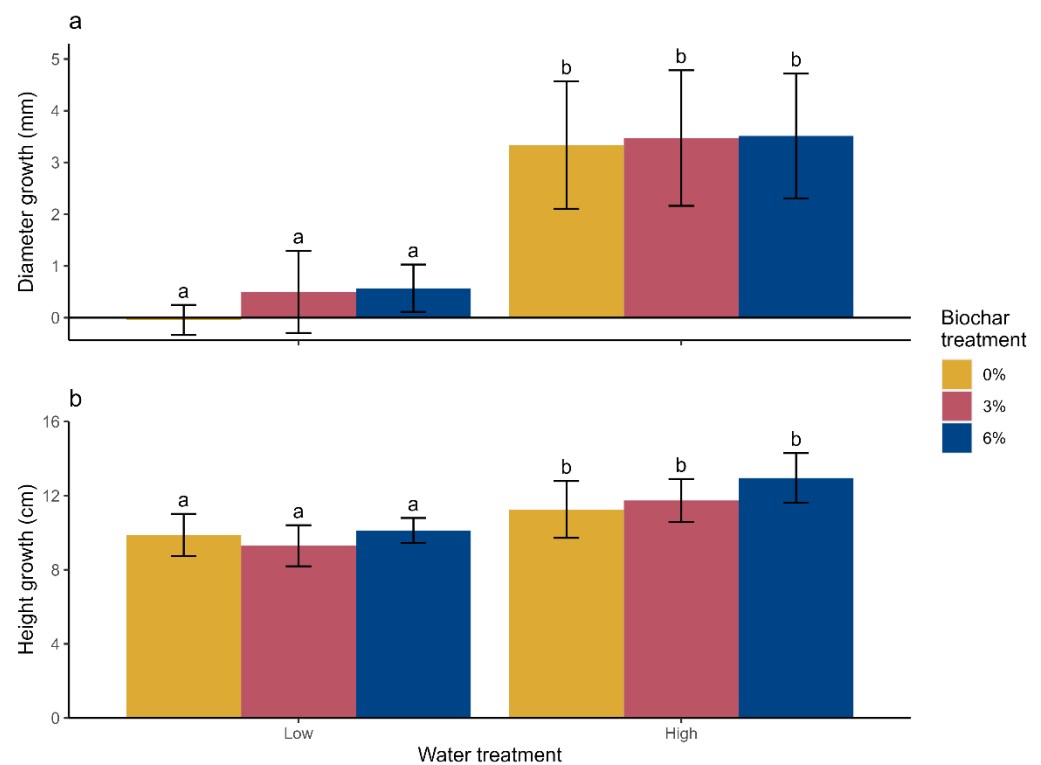


Figure 3. Mean change in seedling diameter (a) and height (b) from planting to end of study by water treatment and biochar treatment. Water treatments had a significant ($p < 0.001$) effect on both diameter and height growth (letters indicate significant differences between water amount treatments); effects of biochar treatment were not significant at $p < 0.05$. Error bars indicate 95% confidence intervals; $n = 15$ for all treatment combinations except for 0% biochar treatments for height growth analysis ($n = 14$ for each water treatment).

Table 3. F-statistic probabilities for fixed effects of measurement week, water treatment, biochar rate, and their interactions on seedling physiology measurements (Photo = photosynthetic rate, Trans = stomatal transpiration rate, and Cond = stomatal conductance rate). Asterisks (*) indicate interactions between terms. Significant p -values ($p < 0.05$) are shown in bold.

Model Term	Num df	Photo <i>p</i> -Value	Trans <i>p</i> -Value	Cond <i>p</i> -Value
(Intercept)	1	<0.0001	<0.0001	<0.0001
Week	6	<0.0001	<0.0001	<0.0001
Biochar	2	0.1292	0.7237	0.6806
Water	1	<0.0001	<0.0001	<0.0001
Week * Biochar	12	0.7719	0.9668	0.83
Week * Water	6	0.1484	0.394	0.0075
Biochar * Water	2	0.6529	0.9517	0.8995
Week * Biochar * Water	12	0.9897	0.9924	0.9414

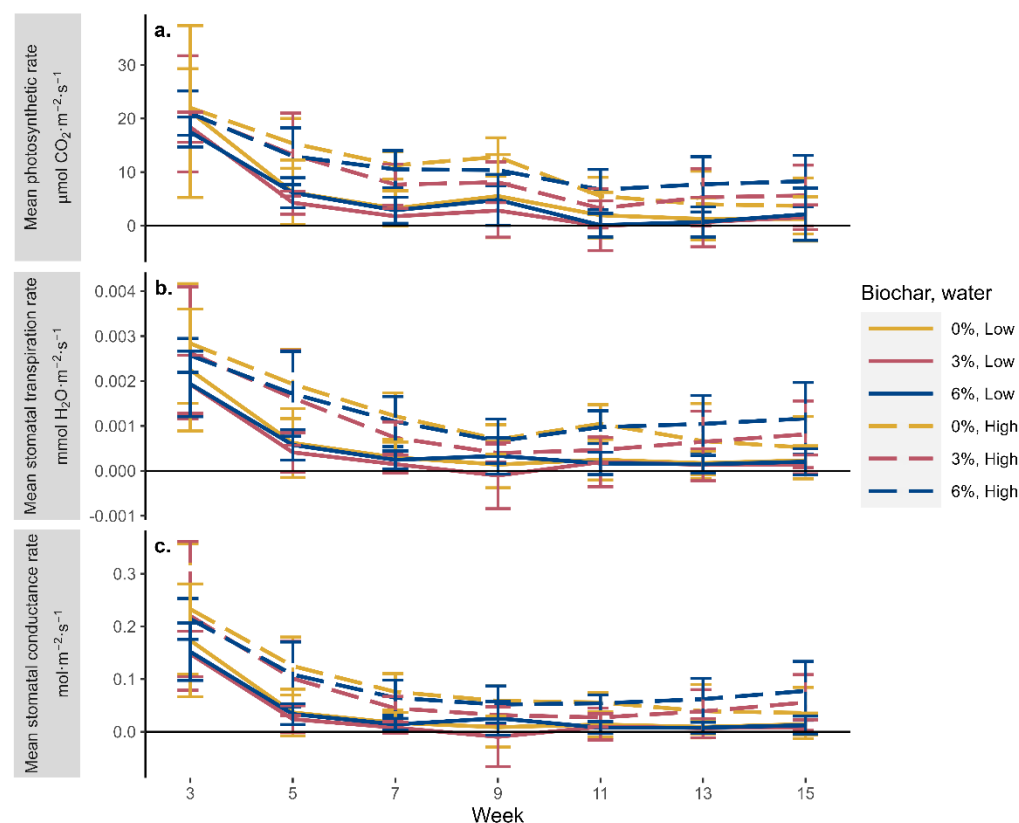


Figure 4. Mean photosynthetic rate (a), stomatal transpiration rate (b), and stomatal conductance rate (c) by week, water treatment, and biochar treatment. Water treatment and week were significant for all three dependent variables; biochar effect was not significant for any. Error bars indicate 95% confidence intervals. See Table S2 for sample sizes.

4. Discussion

Our findings did not support the hypothesis that the addition of biochar to sandy outwash soils will help jack pine seedlings to survive drought or affect seedling growth under low water conditions. We did not see a significant effect of biochar on seedling survival, growth, or physiological measurements in either the drought onset experiment or the water amount experiment.

Many studies have shown promise for the addition of biochar to increase soil water-holding capacity or soil moisture, but the type and condition of biochar that is used, the application method, and the soil type all have important implications for experimental results [31]. “Biochar” is a broad term used to describe a range of products made from different source materials and using different methods. The source material, pyrolysis method and temperature, and biochar particle size may all have impacts on the effects of biochar as a soil additive [50]. The condition of biochar also seems to play an important role in its impact; fresh biochar may be hydrophobic but becomes more hydrophilic after being exposed to soil, air, and/or water [7,9,10]. Several studies have shown that the addition of biochar increased the water holding capacity or moisture of sandy soils [7,8,51], but application methods have also varied among experiments and may have affected results. Page-Dumroese et al. found that biochar mixed into soil repelled water less than surface-applied biochar [11], while a field experiment found that biochar only affected seedling survival when it was applied to the soil surface (biochar mixed into the soil had no effect) [14]. In our experiment, fresh biochar was mixed with soil, and seedlings were planted in it immediately; the results may have been different in both experiments had the biochar been incorporated into the soil for some period prior to planting (e.g., the year prior) or applied only to the soil surface.

Most biochar research at this point has been performed in agricultural settings, in which “low quality” soils are often more nutrient rich and have higher water-holding capacity than “high-quality” forest soils. The soil mixtures used in this study were extremely sandy (89–95% playground sand by weight), likely having lower water holding capacity than sandy soils in the field. In addition to the extreme soil conditions, the drought treatments in these experiments were also extreme (only 20 mL every other day in the low water treatment). We had assumed that any effect of biochar on water availability would more apparent under these extreme conditions, but it is possible that the resulting drought stress was too great for the biochar to have any beneficial effect. Additional work with more organic soils that are considered “richer,” but not as rich as conventional agricultural soils and/or less extreme drought conditions could be performed to test this idea.

One finding from this study was that water treatment had a much stronger effect on seedling diameter growth than on height growth. In the water amount experiment, the mean final height of seedlings in the low water treatment was 19% shorter than seedlings in the high water treatment, but the mean final diameter was 90% smaller in the seedlings receiving low water. Similar results have been found in other studies in which drought treatments had significant effects on the diameter of *Pinus* seedlings but not on height growth; these studies generally attributed this finding to most of the seedlings’ height growth occurring at the beginning of the experiment before drought treatments were started [52,53]. This was likely also the case in our study, as 70–80% of the seedling height growth occurred before the drought treatments were implemented in week 3.

We also found that all seedling physiology measurements (photosynthetic rate, stomatal transpiration rate, and stomatal conductance rate) declined with time throughout the experiment, with sharp drops in each measurement between weeks 3 and 5 (Figure 4). We did not expect to see this drop for seedlings receiving the high water treatment, as other experiments have shown these physiological measures to increase from spring to summer under normal conditions [54] but to decrease under drought conditions [54,55]. We do not believe that the seedlings in the high water treatment were water limited, as they received a large amount of water (200 mL every other day), the soil remained damp throughout the experiment (L. Reuling, personal observation), and the seedlings in this treatment group did not appear wilted (Figure S1). Because they needed to be removed at the end of the experiment to correct for leaf area, the same needles were used for physiology measurements in the LI-6400 for each measurement period and were held together with a bobby pin (Figure S1). It is possible that these needles may have been damaged by the bobby pin or by repeatedly being manipulated to fit into the LI-6400. Additionally, the needles used for measurements were one-year-old, mature needles, many of which had the tips begin to turn brown as the number and size of new growth needles increased. Different study methods may have resulted in a smaller effect of time on physiology measurements, especially in the high water treatment.

There are several reasons why forest and natural resource managers may consider including biochar additions within a forest management plan, aside from attempting to increase seedling drought tolerance. As mentioned previously, in certain instances, the addition of biochar to soil has been shown to increase seedling growth or biomass [14,15,21]. Biochar has also been shown to reduce the allelopathic effects of certain tree species and allow for the growth of other species [56]. Finally, separate from any effect it may have on seedlings, biochar may be seen as part of a natural climate solution because it breaks down extremely slowly and therefore can store carbon over the long term [57,58].

5. Conclusions

We did not find any impact of biochar on seedling growth or survival under drought conditions. While the addition of biochar to sandy soils may not help jack pine seedlings to survive drought, our results suggest that biochar may be added to soil for carbon storage without having negative short-term impacts on seedling growth or survival. However, there are many other potential implications of biochar as a soil addition that are currently being

explored or should be explored, such as the impacts on water, runoff, insects, wildlife, and plant community composition; cost-effectiveness; and more, before biochar is considered a carbon storage and climate solution [59].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijpb14030060/s1>, Table S1: Dry weights of soil mixtures used in both experiments. Table S2: Sample size values (n) for physiology measurements by week. Since some seedlings died throughout the experiment, sample size decreased over time for some treatments. Figure S1: Photo series of seedling 277, which received the high water treatment in the water amount experiment.

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