

# Can Biochar Come to the Rescue of Coastal Barren Species? A Controlled Study Reports on the Impact of Biochar Amendment on Their Survival

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Indigenous species in coastal barren communities are subject to anthropogenic and environmental pressures; some species are in decline, and there is uncertainty about their long-term survival. The authors added supplemental soil carbon in the form of red oak biochar to calcined clay (1:9) to determine the effect of this treatment on survival of legume (*Lupinus perennis* and *Baptisia tinctoria*) and non-legume (*Vaccinium angustifolium* and *Quercus ilicifolia*) species during a period spanning two and a half seasons of unirrigated pot tests. Red oak biochar used in the experiment was produced from pyrolysis, the thermochemical devolatilization and carbonization of the starting biomass. Biochar significantly affected the survival rates of all species ( $P < .03$ ). Biochar-treated non-legumes had higher survival rates ( $P < .10$ ) than similarly treated legumes. Future investigations of biochars, particularly those evolved from recycled lignocellulosic wastes, associated with survival, should focus on reversal of habitat loss.

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## INTRODUCTION

Plant survival in narrow-niche, coastal pine barren communities, imbued with infertile, sandy soils (Orwig *et al.* 2013), grows increasingly problematic (Howard *et al.* 2005) due to stressful anthropogenic (Bertness *et al.* 2002), phenologic (Primack and Miller-Rushing 2011), and climatic (Pennings *et al.* 2005) factors. This investigation sought to determine whether supplemental soil carbon in the form of biochar in a controlled study affects survival rates of several coastal barren species.

Biochar, or *terra preta* (low-temperature black) soil, was first created thousands of years ago (German 2003; Lehmann *et al.* 2011) through a process of burning vegetation under large leaves at high heat under oxygen-depleted conditions. Nowadays, biochars are produced from a variety of devices including top-lit updraft (TLUD) and retort devices (McLaughlin *et al.* 2009). Biochars produced in this way offer advantages as soil additives over other non-pyrolytic organics, such as compost, including higher nutrient content (DeLuca *et al.* 2009; Angst and Sohi 2012), greater soil organic carbon content (Steinbeiss *et al.* 2009; Kimetu and Lehmann 2010; Landesman and Dighton 2010; Major *et al.* 2010), increased soil moisture (Nigussie *et al.* 2012), greater surface area (Zheng *et al.* 2010), and enhanced soil properties (Unger and Killorn 2011).

There has been no research reporting the influence of biochar on coastal pine barren species horticulture, reintroduction of these species, or survival. To examine the survival aspect in a controlled study, we test three hypotheses: there is (1) no difference in survival based on biochar soil amendment; (2) no difference in survival based on species type; and (3) no difference in survival based on legume and non-legume typology.

## EXPERIMENTAL

### Materials

The authors selected four coastal plain species based on similar biomass and classified them as either legume or non-legume. Species were grown from seed or cuttings collected in south central MA a year prior to the start of the test and potted in Waltham, MA (42° 23'04.82" N, 71° 12'50.98" W). Twelve replicates of each of four species were transferred to 1.5 or 3 L PVC nursery pots (Dillen, Myers Industries, Middleboro, OH), depending on amount of biomass. Two media types constituted the pot soils. The first was a calcined clay (Garick Corporation, Chicago, IL) chosen to replicate characteristics of a coastal plain Entisol. It was sieved to a uniform sub 7 mm fraction. The second was red oak biochar blended with the clay; anecdotal evidence suggested biochar enhanced moisture and nutrient. We hypothesized these would play a role in controlled survival experiments.



**Fig. 1.** Clockwise: legumes *Lupinus perennis* and *Baptisia tinctoria* and non-legumes *Vaccinium angustifolium* and *Quercus ilicifolia*

Red oak biochar was produced from the thermochemical conversion of red oak reduced to roughly 10 cm x 20 cm pieces in a single-batch Adam-style retort (designed by Chris Adams, Ethiopia) to 450 °C (high treatment temperature). Conversion consists of devolatilization and carbonization of the starting biomass. The retort is a two- part gasifier

that provides the heat indirectly to the pyrolyzing biomass, which is contained in a separate chamber or retort where the biochar is created.



**Fig. 2.** Left: calcined clay; Right: calcined clay with red oak biochar added

### Biochar Preparation

Following pyrolysis, biochar was air-cooled for 24 h and mechanically pulverized to sub-8-mm particles. Biochar amendment preparation fell into three stages: conditioning, charging, and inoculation. To begin conditioning, raw biochar and dried, ground red oak leaves were saturated. 15 L of biochar particles were placed in a 40 L plastic tub and saturated with 13 L water. In this step, saturation drove off tars, sugars, ash, and lime from the biochar. In a separate tub, an equal volume of leaves was saturated. As the wetted biochar and leaves were solubilized, mycorrhizal activity was encouraged. After a week, as part of a charging process materials were mixed into a single slurry 1:1 and poured into two 40 L tubs. The charging process enabled equilibration of inorganic nutrient and trace elements. In the third week, 75 mL Peters 5-10-5 fertilizer was mixed into each tub. The fertilizer inoculation added additional nutrient to increase available nutrient, which was converted to sugars fed on by microbes. It should be noted that as sugars were driven from the biochar through microbial action, mobile matter became leachable carbon, accompanied by an increase in water uptake. In the fourth week, the curing process continued; each slurry mixture was removed from the tubs and shoveled onto a wire mesh attached to a wooden frame with the screen suspended over two empty 20 L tubs. Approximately 2 L of water were drained from the slurries, after which the remaining non-liquified material was removed and air dried in a separate dry container. By week five, sufficient drying had occurred for the biochar to be blended with the clay mineral. For biochar amended clay media, the blend ratio was 1:9 red oak biochar to clay. Previous findings have indicated optimum results for biochar addition should fall between 10-12% volume (Yu *et al.* 2013). After potted materials were arranged randomly on an experimental platform, they were watered to 100% field capacity, then left unirrigated through the balance of the experiment which progressed over two and a half growing seasons.

### Analytic Methods

Clay nutrients were analyzed using a Modified Morgan solution employing inductively coupled plasma atomic emission spectrometry (ICP-AES) (Perkin Elmer Elan 5000; Waltham, MA) analysis. The Modified Morgan's (MM) solution, a 1.25 M

ammonium acetate buffer at pH 4.8, is a standard used by university soil laboratories in the Northeast U.S.

The biochar sample was milled to a uniform 0.25 mm to 0.5 mm fraction and rinsed with sterile water to remove fine ash, then prepared using a SPEX mill grinder and weighed to  $\pm 5$  mg on a Sartorius M2B analytical microbalance (DWS Inc.; Elkington, IL). Several standard methods of soil analysis were modified to account for biochar properties such as buoyancy and hydrophobicity, which make the centrifugation and wetting of biochar challenging. Due to a high initial pH, the Modified Morgan's (MM) solution was used to assess biochar CEC. Phosphorus concentrations in the extracts were determined using the ascorbic acid method on a Lachat Quickchem 8500 flow injection (high sample throughput and rapid method changeover) analyzer (Lachat Instruments; Loveland, CO). Concentrations of available macro mineral elements (K, Ca, Mg, and Na) and micro mineral nutrients (Zn, B, Cu, Fe, and Mn) in the soils were determined by extraction with a MM solution followed by inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis (Perkin Elmer Elan 5000; Waltham, MA). Soil pH was assessed by measuring the proton ( $H^+$ ) activity of a soil/water (1:1) slurry with a pH meter.

Other analyses were performed, following methods described by Amlinger *et al.* (2004), Enders *et al.* (2012), and Rayment and Higginson (1992). A muffle furnace (SSFE, Davis Instruments) was used to measure ash content using a crucible sample heated to 550 °C until all combustible organic carbon was removed. C, H, N, and O were determined by ultimate analysis (ASTM D-3176-09) using a Perkin Elmer 2400 CHNS/O continuous flow isotope ratio mass spectrometer and elemental analyzer. A second sample, cardboard biochar, was prepared using the same methods.

Adsorption was assessed using a gravimetric adsorption capacity scan (GACS) method (McLaughlin *et al.* 2012). A sample wire basket containing a biochar sample was heated to 300 °C under nitrogen purge, during which the sample was stabilized by the removal of any adsorbed moisture and low boiling volatiles. At 300 °C, the heating supply was turned off and the purge gas surrounding the sample changed to pure R134a (a refrigerant used in air conditioning systems adapted to this method). Adsorption data was calculated as the percent weight gain of R134a per minimum weight of the char sample over the entire temperature history.

## RESULTS AND DISCUSSION

In Table 1, results indicate that calcined clay yielded more than twice the K and Mg fertility of clay treated with biochar. Ca values were abnormally high due to the influence of carbonates. P availability was greater in the amended clay; this was an important clue as to the presence of higher water holding capacity; a higher P value is known to correlate with increased moisture retention (Uzoma *et al.* 2011). Trace element levels were somewhat lower after biochar amendment; Beesley *et al.* (2011) reported reduced trace metals in a field trial using biochar and greenwaste. Biochar CEC ( $76 \text{ cmol+}/\text{kg}^{-1}$ ) was found to have an effect on amended calcined clay CEC ( $15.2 \text{ cmol+}/\text{kg}^{-1}$ ) when compared with calcined clay only CEC ( $5.4 \text{ cmol+}/\text{kg}^{-1}$ ). One year later, a retest of CEC found amended clay had increased to  $19.5 \text{ cmol+}/\text{kg}^{-1}$ . As a reliable indicator of soil health, soil cation exchange capacity (CEC) measurement is preferred over biochar CEC measurement,

which relies on internal ion exchange; soil CEC is dependent on soil surface (external) ion exchange.

**Table 1.** Initial Fertility Characteristics using Modified Morgan's Solution

	Calcined clay	Calcined clay/ red oak biochar	Red oak biochar
Dry weight	3.5 g/5cc	3.3 g/5cc	1.4 g/5cc
% OM	6.5	6.9	8.2
pH	6.1	6.6	7.6
		expressed as ppm	
P	13	111	492
K	76.5	194	626
Ca	651.6	2026	2535
Mg	67.9	138	391
B	1.3	1.1	4.8
Mn	6.8	6.1	11.4
Zn	7.3	3.6	3.9
Cu	.2	.3	.2
Fe	6.3	2.5	3
S	30.4	412	72
Pb	29	27	27

In Table 2, one year follow-up results of Modified Morgan extract tests were performed. Most macronutrient, OM, pH, and trace metal levels were lower; however, high calcium carbonate was still very much in evidence. P cation availability decreased, consistent with expectations of leaching effects.

**Table 2.** One Year Fertility using Modified Morgan's Solution

	Calcined clay	Calcined clay/ red oak biochar	Red oak biochar
Dry weight	3.5 g/5cc	3.1 g/5cc	1.3 g/5cc
% OM	5.7	9.9	5.3
pH	5.9	6.9	7.4
		ppm	
P	10	53.2	89
K	55	268	866
Ca	420	3033	1863
Mg	81	308	198
B	1	1	2.1
Mn	7	6.1	11.4
Zn	9	7	4.8
Cu	.01	.4	.3
Fe	5	3.6	1
S	30.2	15.8	31
Pb	22	5	16

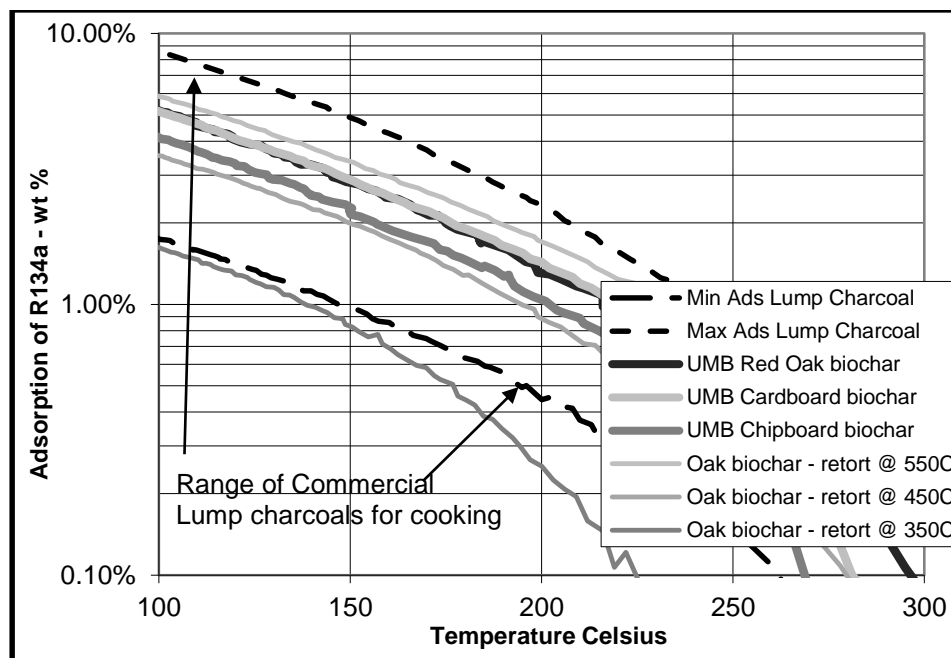
Besides evaluating biochar effect on nutrient, we desired to gain some perspective on elemental analysis of two converted, homogeneous feedstocks, red oak and cardboard. C, H, N, and O properties were compared, as can be seen in Table 3. Previous findings reported by Mukome *et al.* (2013), Basso *et al.* (2013) and Mitchell *et al.* (2013) shed light

on elemental properties of red oak biochar; the current study is the first to compare red oak and cardboard biochar properties using these methods to further understand the contrast between wood-based and paper-based recycled chars. In this study we were also interested in comparing carbon/nitrogen ratios, as high C/N values (>1.5 or 150) have been determined to be an indicator of attractive moisture capacity (Cardosa *et al.* 2013). In this study C/N ratios were substantial, although measurements were somewhat skewed because of difficulties in obtaining precise measurements of biochar productivity. O/C ratios were another interest. These are shown in Table 3; O/C ratios below 0.4 are considered attractive for promoting mycorrhizal activity while slowing mineralization (Spokas 2010), which allows total organic C to be maximized over time. Moisture capacity may be a determinant of survival tendencies.

**Table 3.** IBI Level 1 Proximate Analysis

	Red oak biochar	Cardboard biochar
Yield (%)	20	25
Ash (%)	1	14.4
C (%)	84.7	72.1
H (%)	5.3	4.8
N (%)	.5	.24
O	8.5	8.2
O/C	10	11.3
C/N	169.4	300.4
Moisture (%)	1.8	4.1
AR (%)	6.9	5.2

Still another test, the Gravimetric Adsorption Capacity Scan test (GACS), was performed to compare biochar adsorption rates. Results are shown in Fig. 3.



**Fig. 3.** GACS analysis showing R134a capacity for wood and non-wood feedstock biochar

The GACS test measures the difference in R134a adsorption capacity for the experimental and other biochars. It is used to obtain a maximum global comparison of adsorption data for red oak and cardboard biochars compared with other, benchmark chars. Adsorption is measured by providing a “challenge gas”, in this case R134a, and measuring the weight gain under a range of temperatures corresponding to a range of adsorption energies. Insight gained from the R134a results can be used to make educated guesses about potential increases in soil moisture and water vapor affinity due to the biochar addition. Adsorption of the challenge gas correlates with the ability of the biochar to retain moisture and soluble organics in soils. Our understanding of biochar water dynamics is based on analogous behavior of activated C with respect to water vapor adsorption.

## SURVIVAL ANALYSIS

A survival analysis was performed using Kaplan Meier (KM) curves in R, a test for differences in group survival rates is an estimate using a non-parametric maximum likelihood estimator known as the Kaplan-Meier estimator. Survival probability is estimated by specifying a survival function for the data. In this experiment we modeled a number of species as a function of time depending on treatment type (clay or biochar modified clay). The model was then estimated using maximum likelihood, giving a fitted probability of survival for each time period. The model was further enhanced by applying the Kaplan-Meier estimate of the survival function. The plot of the KM estimate of the survival function is a series of horizontal steps with declining magnitude.

## SURVIVAL RESULTS

At the 5% level of significance ( $P < .030$ ), the authors rejected the first null hypothesis and concluded red oak biochar and untreated calcined clay did not produce the same survival rate. At each milestone of the 15-month test, which covered a period of two and a half growing seasons, inoculated species outperformed their non-treated counterparts, as shown in Fig. 4. *Quercus ilicifolia* was the only one of the four species that showed only a modest survival benefit (15% compared with the other treatment) from biochar. To compare the effect of calcined clay and red oak biochar blended with calcined clay on survival rates, a survival plot was used to represent the difference in mean survival found to be statistically significant at 5% level when plant subjects were treated to biochar-added soil. Survival plots in Fig. 4 display 95% confidence bands (dotted lines) in the plots overlap, suggesting no significant difference at a given time period. However, the log-rank test asks whether the two survival curves are different across all time periods, which is highly relevant to our analysis. The results show a statistically significant difference for biochar vs. calcined clay ( $p = .030$ ), and legume vs. non-legume ( $p = .10$ ).

A simultaneous test was performed for mean survival across all time periods. In Fig. 5, 95% confidence bands are shown for each individual time period. This explains why the 95% confidence bands can overlap (suggesting no significant difference in survival rate), but the joint test showed a significant difference. Bands (dotted lines) also

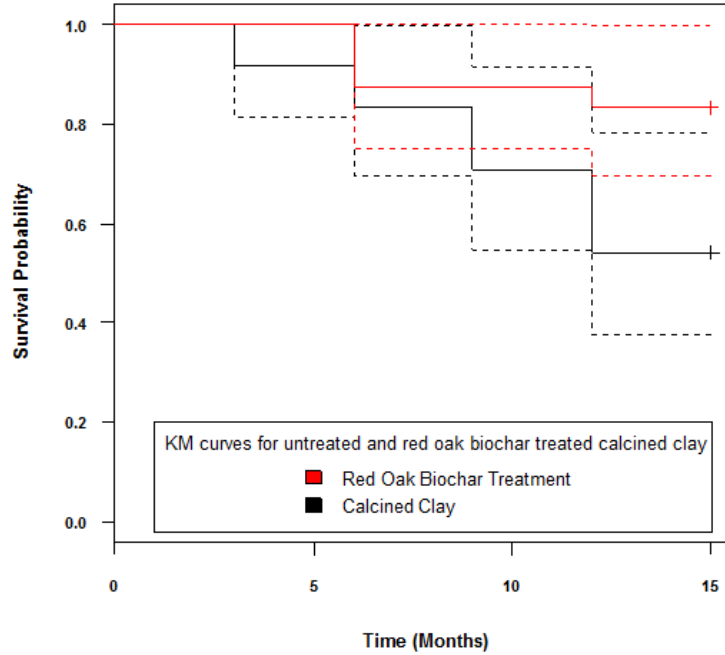


Fig. 4. Effect of biochar treatment on overall species survival. KM curves for untreated and red oak biochar treated calcined clay

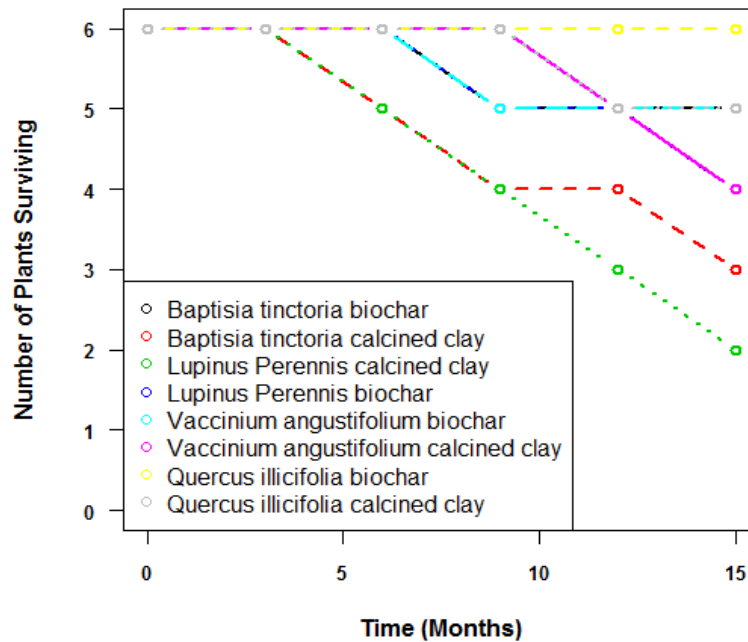


Fig. 5. Times series plot shows overall species survival depending on treatment type

represent the sampling variability of the data. Because the sample data represent only a subset of the whole population, the true population survival rate will be somewhat different, and the confidence bands represent this uncertainty. Species-soil time series plots (Fig. 5)



portrayed a sizable difference in survival by the 15-month mark. However, the sample sizes were too small to produce statistically significant differences regarding species-to-species relationships. Thus, the null hypothesis was accepted.

As Fig. 6 illustrates, non-legumes had a significantly higher survival probability ( $P < .10$ ) than their legume counterparts. Like results reported in Fig. 4, those in Fig. 6 show 95% confidence bands (dotted lines) in overlapped plots, suggesting that differences in survival curves were evident across all time periods. Enhanced legume survival was an important milestone in this study, given the difficulty of transplanting legumes to pots or field placement (Smith 1997).

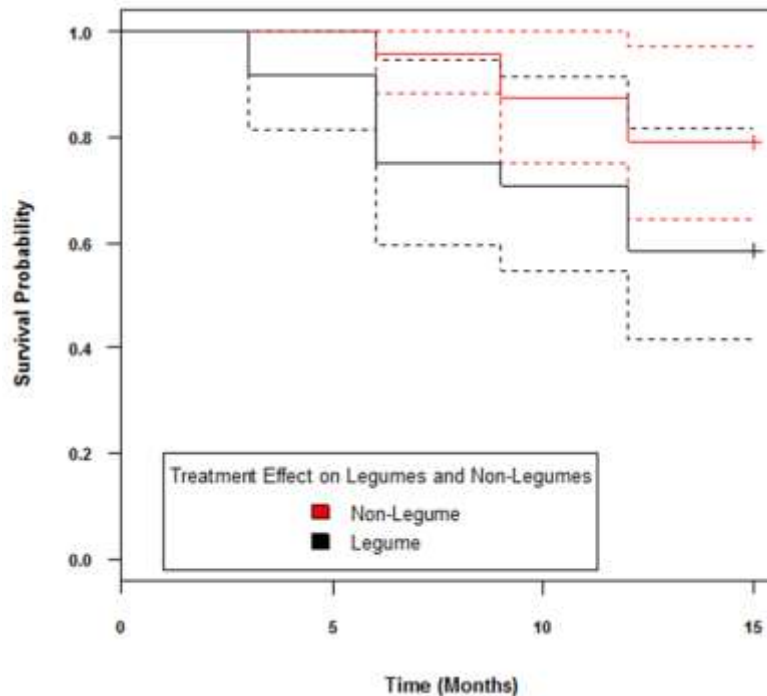


Fig. 6. Effect of biochar treatment on legume versus non-legume survival

## CONCLUSIONS

Red oak biochar elevated survival rates of a number of coastal barren species under controlled conditions; this in itself is a finding that suggests further investigation of biochar supplement to augment survival of species in other biome types. Still, a number of pressing questions remain. For example, will increasing specie sample sizes reveal more decisive data regarding species-to-species comparisons of biochar-based survival benefits? If one hypothesizes, as we did, that there is a connection between survival and moisture retention following biochar amendment, is there evidence the biochar phenomenon extends past the limits of our controlled study? With regards to this last question, there is a growing body of evidence suggesting that biochar enhances soil moisture and nutrient holding for low fertility Ultisols (Novak *et al.* 2009) from the Southeast U.S. and soil moisture retention, shrinkage and plasticity for four different Entisols (Licht *et al.*, in preparation) found in Northeast U.S. coastal locations. Further investigation may determine a connection

between soil characterization (*i.e.*, amended soils with elevated nutrient and water holding capacity), characterization based on biochar blend type and plant physiological response such as water use efficiency. Some preliminary data has been gathered to demonstrate changes in water use efficiency as a result of increased carbon from red oak and cardboard biochar soil amendment (Licht *et al.*, in preparation). However there is much to learn about the relation between carbon and water use efficiency of species in infertile soils and whether or not biochar feedback is merely temporary.

Before biochar of any kind is tested *in situ*, more work is required to understand current findings under the lens of feedstock selection, blending technique, and long-term effects (Gurwick *et al.* 2013). As feedstock selection widens, for example, use of recycled waste from paper products (Mitchell *et al.* 2013) may add to value already seen from wood sources (*e.g.*, red oak, spruce-pine-fir), dung (Guo *et al.* 2014), crop residues (Yuan and Xu 2011), or wildlife-produced charcoal from forest fires (MacKenzie and Drozdowski 2013). We compared cardboard, an homogenous feedstock, to red oak, another homogenous feedstock, to better understand the similarities or differences between these converted waste biochars. Certainly one important research benefit from its use as a research tool rests in the knowledge that cardboard feedstock, whether corrugated, fiberboard or chipboard, is replicable worldwide.

Perhaps the issue that is most central to the potential value of biochar as an ecological tool lies in determining whether biochar benefits will extend survival of other species in other plant communities or, for example, in disturbed plant communities such as those found on former military firing ranges (Moon *et al.* 2013; Uchimiya and Bannon, 2013) where mitigation is an additional concern. If biochar can be shown to improve soil fertility and enhance plant physiological response even after anthropogenic disturbance, perhaps it can play a rescue role in revitalization efforts to reverse habitat loss in the Northeast U.S. (Foster and Motzkin 2003).

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