Tropical Tree Species Effects on Soil pH and Biotic Factors and the Consequences for Macroaggregate Dynamics
First-Year Vitality of Reforestation Plantings in Response to Herbivore Exclusion on Reclaimed Appalachian Surface-Mined Land

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Received: 30 March 2018; Accepted: 19 April 2018; Published: 21 April 2018

Abstract: Conventional Appalachian surface-mine reclamation techniques repress natural forest regeneration, and tree plantings are often necessary for reforestation. Reclaimed Appalachian surface mines harbor a suite of mammal herbivores that forage on recently planted seedlings. Anecdotal reports across Appalachia have implicated herbivory in the hindrance and failure of reforestation efforts, yet empirical evaluation of herbivory impacts on planted seedling vitality in this region remains relatively uninitiated. First growing-season survival, height growth, and mammal herbivory damage of black locust (Robinia pseudoacacia L.), shortleaf pine (Pinus echinata Mill.), and white oak (Quercus alba L.) are presented in response to varying intensities of herbivore exclusion. Seedling survival was generally high, and height growth was positive for all species. The highest herbivory incidence of all tree species was observed in treatments offering no herbivore exclusion. While seedling protectors lowered herbivory incidence compared with no exclusion, full exclusion treatments resulted in the greatest reduction of herbivore damage. While seedling protectors lowered herbivory incidence compared with no exclusion, full exclusion treatments resulted in the greatest reduction of herbivore damage. Although herbivory from rabbits, small mammals, and domestic animals was observed, cervids (deer and elk) were responsible for 95.8% of all damaged seedlings. This study indicates that cervids forage heavily on planted seedlings during the first growing-season, but exclusion is effective at reducing herbivory.

Keywords: mine reclamation; browse; black locust; shortleaf pine; white oak; elk; white-tailed deer; rabbit; small mammal

1. Introduction

Surface mining for coal has negatively impacted forest resources across Appalachia, including the loss of over 1.1 million ha of forests [1] and the fragmentation of at least an additional 1 million ha [2,3]. Federal regulations of the Surface Mining Control and Reclamation Act of 1977 (SMCRA) led to reclamation methods that, while intended to limit soil destabilization and water-quality impairment, resulted in compacted post-mining landscapes that greatly hinder forest regeneration. Compacted mine soils inhibit water infiltration, increase the frequency of ponding, and suppress root spreading [4–6], which diminishes water and nutrient absorption and root anchoring ability critical for vertical stability with tree maturation [7]. Post-mining vegetation communities in Appalachia are typically composed of planted invasive, exotic woody and herbaceous species (e.g., autumn-olive (Elaeagnus umbellata Thunb.), sericea lespedeza (Lespedeza cuneata (Dum. Cours.) G. Don), and multi-flora rose (Rosa multiflora Thunb.)) that rapidly colonize disturbed areas and outcompete native pioneer species [8–10]. Additionally, intensive vegetation control in popular agricultural post-mining land-uses, such as hayland pasture and crop production, can preclude forest succession and reforestation efforts.
Motivated by the exigencies of mine reforestation under conventional reclamation standards, a multi-disciplinary group of investigators initiated a large-scale study of techniques that would improve the favorability of post-mining landscapes for reforestation [11]. The Forestry Reclamation Approach (FRA) advocates a broad five-step method for mine reforestation that includes site preparation to create adequate rooting media and the use of proper tree planting techniques [11,12]. Heavy machinery is typically used to reduce pre-existing competing vegetation and alleviate soil compaction to create proper rooting media for planted seedlings. Restoration of native forests on reclaimed mined lands is reliant upon artificial regeneration. Distance to native seed sources, absence of soil seed bank, and abundant seed availability from non-native invasive species often hinder natural regeneration and necessitate tree planting to commence forest growth. However, after planting, seedlings are subject to a variety of factors that can decrease survival, growth, and subsequent forest maturation, of which herbivory can be among the most impactful.

Herbivory can greatly influence vegetation communities. Individual plant factors, such as species, life stage, nutrient quality, and defensive chemical potency [13–16], contribute to the extent of herbivore damage to plant communities. Community-level impacts, including floral dynamics [17,18], herbivory timing and intensity [19,20], and trophic interactions [21,22], also dictate the influence of herbivory. The loss of apex predators in the eastern U.S. has aided in the overabundance of primary consumers, specifically white-tailed deer (Odocoileus virginianus Zimmermann), a species noted for its impact on plant composition and structure in eastern U.S. ecosystems, including the biodiverse mixed-mesophytic forests of Appalachia [23]. Vulnerable plants, such as American ginseng (Panax quinquefolius L.) and several understory forbs, have experienced sharp declines in numbers and population viability as a result of increased deer browsing [14,24,25]. Areas with high deer densities commonly experience regenerating forests with compositions reflective of differences in plant species palatability and defensive mechanisms to reduce browsing; less palatable and more defensive plant species become more common in these areas, which dictates compositional and structural changes manifested with forest aging [26].

Herbivory can be particularly detrimental to newly established tree plantations. Artificial regeneration is often selected to alter pre-existing cohort species compositions, to reforest (or afforest) a non-forested area, and/or to accelerate the rate of regeneration. Therefore, plantation failure can prove both ecologically and financially costly, especially to highly denatured surface-mined lands where tree planting is vital to successful reforestation. Recently, herbivore damage of reforested seedlings has been implicated in the widespread damage to several FRA plantings across Appalachia [27]. However, aside from anecdotal claims and isolated information in a few published studies [28,29], a formal investigation of herbivory impacts on mine reforestation remains lacking. We present the first empirical study of herbivory damage to tree seedlings planted under the FRA on reclaimed Appalachian mined lands. We examined survival, height growth, and relative cause-specific herbivory of black locust (Robinia pseudoacacia L.), shortleaf pine (Pinus echinata Mill.), and white oak (Quercus alba L.) seedlings in response to herbivore exclusion.

2. Materials and Methods

2.1. Plot Design and Data Collection

We selected four ~0.4-ha sites across a complex of surface-mined tracts owned by the University of Kentucky in Breathitt County, KY, USA (Figure 1). Following FRA site preparation recommendations [12], each of the sites was bulldozed to reduce pre-existing vegetation (primarily invasive, exotic species), and compacted soils were ripped with a ripping shank mounted behind a Caterpillar D-11 bulldozer. Each site was partitioned into three, 36-m square plots, and ~108 1-0 bare-root seedlings of each of black locust, shortleaf pine, and white oak were planted randomly in rows on a 2-m spacing within each plot (4 sites × 3 plots/site = 12 plots). Seedlings were purchased from
the Kentucky Division of Forestry nursery and were planted by experienced reforestation contractors in March 2017.

Figure 1. Study location prior to site preparation, Breathitt County, KY, USA. Exotic shrubs and conifers were common in the two western plot locations, and vegetation was relatively absent in the eastern plots.

Similar to many legacy mined lands across Appalachia, the study site harbors a number of herbivores capable of damaging planted seedlings, including elk (*Cervus canadensis* Erxleben), white-tailed deer, rabbits (*Sylvilagus* spp.), and small mammals. Small mammal communities across study sites were predominantly composed of white-footed mice (*Peromyscus leucopus* Rafinesque) [Z. Hackworth, unpublished data], which is consistent with prior work on adjacent mined lands in eastern Kentucky [30]. The study site also harbors a semi-feral horse (*Equus ferus caballus* L.) population and, occasionally, domestic cattle (*Bos taurus* L.) that have escaped from neighboring properties. Since domestic animal occupancy of abandoned mined lands is common throughout Appalachia and confirmed in our study area, damage caused by this group was included in the analysis. We were only interested in seedling damage mediated by mammal herbivores and did not examine herbivory from other taxa (e.g., insects).

A randomized complete block experimental design was used, whereby each plot within a site was randomly prescribed one of three herbivore exclusion treatments: no exclusion, seedling protectors, or full exclusion. The no exclusion treatment served as the control within a site replicate and offered
unobstructed access to all herbivores. Within plots assigned protector treatments, an 8.5-cm × 46-cm (diameter × height) plastic diamond-mesh seedling protector (Forestry Suppliers, Jackson, MS, USA) was installed around each seedling, the base of which was entrenched in the soil 2–3 cm below the surface, and was anchored with a bamboo stake. Protector plots were designed to exclude small mammals and rabbits, but allow ungulate herbivory. In full exclusion treatments, a 2.4-m fence constructed from treated wooden posts and 12.5-gauge woven wire (Kencove Farm Fence Supplies, Blairsville, PA, USA) was installed around the perimeter of the plot, and each seedling within the plot was surrounded by a seedling protector according to the protocol implemented for protector treatments. Full exclusion was designed to prohibit seedling access to all aboveground mammal herbivores of interest to this study.

Soil samples were collected from all experimental plots to determine variability in edaphic characteristics across the experiment. Each plot was halved longitudinally, and a sample aggregated from three random subsamples was collected from each half of the plot prior to planting. Soil samples were analyzed for the following soil parameters: pH, P, K, Ca, Mg, Zn, N, and exchangeable K, Ca, Mg, and Na. Soil pH was calculated in a 1:1 soil:water solution [31]. P, K, Ca, Mg, and Zn concentrations were extracted via Mehlich III [32]. Relative sand, silt, and clay percentages were calculated with the micropipette method [33]. Exchangeable nutrient concentrations were determined after ammonium acetate extraction with ICP [32]. Total N (%) was evaluated with a LECO CHN-2000 Analyzer (LECO Corporation, St. Joseph, MI, USA). Cation exchange capacity was assessed by the ammonium acetate method at pH 3 [34]. Soil parameter differences among exclusion treatments were compared via a linear mixed-effect model with exclusion treatment as a fixed effect and site as a random effect. Significant differences were evaluated using a Type III ANOVA model. No significant differences among exclusion treatments were observed for any of the selected soil parameters (Table 1).

Table 1. Edaphic characteristics (Mean ± SE) across herbivore exclusion treatments on reclaimed mined lands in southeastern KY. No significant differences among exclusion treatments were detected for any of the soil parameters based upon individual Type III ANOVA models and a 0.05 significance level.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No Exclusion</th>
<th>Protector</th>
<th>Full Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>5.60 ± 0.52</td>
<td>5.62 ± 0.75</td>
<td>6.08 ± 0.69</td>
</tr>
<tr>
<td>P (mg/kg)</td>
<td>5.94 ± 0.91</td>
<td>11.19 ± 6.06</td>
<td>6.00 ± 1.01</td>
</tr>
<tr>
<td>K (mg/kg)</td>
<td>71.06 ± 17.17</td>
<td>65.81 ± 14.32</td>
<td>63.94 ± 12.11</td>
</tr>
<tr>
<td>Ca (mg/kg)</td>
<td>580.13 ± 168.38</td>
<td>653.44 ± 183.04</td>
<td>640.44 ± 163.42</td>
</tr>
<tr>
<td>Mg (mg/kg)</td>
<td>251.19 ± 83.16</td>
<td>225.38 ± 75.81</td>
<td>269.06 ± 73.44</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>3.65 ± 1.35</td>
<td>3.49 ± 1.49</td>
<td>3.49 ± 1.16</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.07 ± 0.03</td>
<td>0.10 ± 0.06</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>52.18 ± 10.14</td>
<td>56.85 ± 8.48</td>
<td>57.25 ± 8.43</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>33.38 ± 7.94</td>
<td>29.10 ± 6.31</td>
<td>39.33 ± 6.24</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>14.44 ± 2.28</td>
<td>14.05 ± 2.37</td>
<td>13.42 ± 2.20</td>
</tr>
<tr>
<td>CEC † (meq/100 g)</td>
<td>7.09 ± 1.25</td>
<td>6.91 ± 1.40</td>
<td>6.65 ± 1.47</td>
</tr>
<tr>
<td>Exch † K (meq/100 g)</td>
<td>0.20 ± 0.05</td>
<td>0.18 ± 0.04</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Exch Ca (meq/100 g)</td>
<td>2.97 ± 1.00</td>
<td>3.11 ± 0.93</td>
<td>3.49 ± 1.01</td>
</tr>
<tr>
<td>Exch Mg (meq/100 g)</td>
<td>1.94 ± 0.74</td>
<td>1.69 ± 0.64</td>
<td>2.17 ± 0.68</td>
</tr>
<tr>
<td>Exch Na (meq/100 g)</td>
<td>0.02 ± 0.003</td>
<td>0.02 ± 0.001</td>
<td>0.02 ± 0.004</td>
</tr>
</tbody>
</table>

* CEC indicates cation exchange capacity. † Exch indicates exchangeable.

First growing-season survival, height growth, and herbivore damage of seedlings were monitored via a series of seedling assessments. In May 2017, each seedling was assessed for survival, and the initial heights of all seedlings were measured. In October 2017, the end-of-growing-season survival of all seedlings was recorded, and the height of all seedlings was remeasured. Seedling heights were measured from the ground line to the tip of the apical bud of the tallest seedling branch. In February 2018, each seedling was evaluated for the presence of mammal herbivory to
assess cumulative herbivory across all seasons. Herbivory indicators were categorized into four cause-specific groups: cervids, rabbits, small mammals, and domestic animals. Elk and deer produced nearly identical browse indicators, and, since one-year-old seedlings were below the “browse line” of both species, herbivory could not be distinguished between them and was, therefore, classified as “cervids”. Cervid herbivory was typically identified by the damage or removal of shoot terminal buds which left a characteristically ragged edge due to the lack of upper incisors and biting of the bottom teeth against the upper lip pad. A clean, angular branch severance near the base of the seedling or complete seedling severance near the ground was attributed to rabbit herbivory. Basal bark gnawing was considered characteristic of small mammal herbivory.

Since herbicide was not employed during site preparation, regrowing competing vegetation could impact seedling survival and growth. However, fencing in full exclusion treatments may produce taller competing vegetation heights due to the exclusion of large herbivores. Height of competing vegetation was measured via ten random subsamples within each experimental plot in October 2018. Mean vegetation height of treatments without fencing (i.e., no exclusion and protector treatments) was 54.2 cm, and mean height of vegetation within full exclusion treatments was 75.9 cm, indicating that fencing promotes higher levels of competing vegetation compared with non-fenced treatments.

2.2. Statistical Analysis

First-year seedling survival, height growth, and herbivory damage were evaluated using a model with species and exclusion treatment as the main effects, a species x treatment interaction term, and site as a random effect. All analyses were performed in Program R 3.4 [35]: generalized linear models were fit using functions in the “lme4” package [36]; overall species and treatment effects were evaluated using a Type III ANOVA model within the “car” package [37]; and differences among levels in significant main effects were calculated with Tukey-corrected pairwise comparisons in the “lsmeans” package [38]. A 0.05 significance level was observed for all statistical tests.

Using survival data collected in the May and October 2017 assessments, first growing-season survival was calculated per the following formula:

\[
\text{Survival} = \frac{\text{Seedlings alive}_{\text{October 2017}}}{\text{Seedlings counted}_{\text{May 2017}}}.
\] (1)

To elucidate plot-level survival differences among tree species and herbivore exclusion treatments, survival was tested as the response variable in a mixed-effect generalized linear model using the binomial distribution and logit link function.

With seedling height data collected in the May and October 2017 assessments, plot-level height growth of live seedlings was calculated per the following formula:

\[
\text{Growth} = \text{Mean Height}_{\text{October 2017}} - \text{Mean Height}_{\text{May 2017}}.
\] (2)

Height growth was analyzed using a linear mixed-effect model, and a natural logarithmic transformation of growth was used as the response variable to satisfy model assumptions.

With February 2018 seedling data, the proportion of herbivory-damaged trees was calculated as:

\[
\text{Herbivory} = \frac{\text{Seedlings damaged}}{\text{Seedlings assessed}}.
\] (3)

Herbivory was first modeled via a mixed-effect generalized linear model using a binomial distribution and logit link function with species and exclusion treatment as the main effects, a species x treatment interaction, and site as a random effect. However, due to model non-convergence, the random effect was removed, and the model was refit with only fixed effects.
3. Results

3.1. Survival

Survival estimates from the first growing-season (May–October) are presented in Table 2. A significant interaction was observed for mean survival between tree species and exclusion treatment ($\chi^2 = 28.6$, $p < 0.001$). Black locust demonstrated higher mean survival in protector (80.3%) and full exclusion (81.7%) treatments compared with no exclusion treatments (73.1%). Shortleaf pine survival was low across all treatments: while mean survival was similar in no exclusion (37.8%) and protector (36.5%) treatments, and shortleaf pines in full exclusion plots exhibited lower survival (28.5%). White oak survival was higher in protector (80.5%) and full exclusion (80.5%) treatments compared with no exclusion treatments (68.2%). In no exclusion treatments, black locust and white oak survivals were higher than that of shortleaf pine. Similarly, in protector and full exclusion treatments, no significant differences were present in black locust and white oak survival; however, survivals of both species were higher than that of shortleaf pine.

Table 2. First growing-season seedling survival (%) Mean ± SE) among tree species and exclusion treatments on reclaimed mined lands in southeastern KY. Means with differing letters indicate significant differences among exclusion treatments within a species, and means with different symbols indicate significant differences among species within an exclusion treatment, as determined via Type III ANOVA and subsequent Tukey-corrected pairwise comparisons at a 0.05 significance level.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species</th>
<th>No Exclusion</th>
<th>Protector</th>
<th>Full Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Locust</td>
<td>73.1b ± 10.6</td>
<td>80.3a ± 6.0</td>
<td>81.7a ± 9.9</td>
<td></td>
</tr>
<tr>
<td>Shortleaf Pine</td>
<td>37.8a ± 10.0</td>
<td>36.5a ± 9.7</td>
<td>28.5b ± 8.7</td>
<td></td>
</tr>
<tr>
<td>White Oak</td>
<td>68.2b ± 10.4</td>
<td>80.5a ± 5.3</td>
<td>80.5a ± 6.0</td>
<td></td>
</tr>
</tbody>
</table>

3.2. Height Growth

The Type III ANOVA model testing for differences in mean height between tree species and exclusion treatments provided little evidence for an interaction ($\chi^2 = 3.5$, $p = 0.463$). After removing the interaction term and refitting the model, tree species ($\chi^2 = 57.0$, $p < 0.001$) and exclusion treatment ($\chi^2 = 10.4$, $p = 0.005$) were found to be significant in predicting height growth. Among tree species, mean height growth of black locusts (30.3 cm) was significantly greater than that of shortleaf pine (11.9 cm) and white oak (8.6 cm); there was no difference in mean height growth between shortleaf pine and white oak (Figure 2). Protector treatments (mean = 20.3 cm) sustained significantly higher mean height growth compared with full exclusion treatments (mean = 13.5 cm; Figure 2). Significant differences were not present between protector and no exclusion treatments (mean = 17.0 cm) or between full exclusion and no exclusion treatments.
no exclusion, - rabbits, cervid white oak seedlings were damaged less frequently (51.1%); and shortleaf pine damage was the least. Within-species trends for herbivore exclusion treatments: within each species, no exclusion treatments contained the highest herbivory percentages, followed by protector treatments, and full exclusion treatments (Table 3). Within no exclusion plots, black locust was damaged most frequently (85.1%); white oak herbivory was significantly lower (72.6%); and shortleaf pine was the least damaged of all species (34.1%). Black locust was the species damaged most often in protector treatments (73.8%); white oak seedlings were damaged less frequently (51.1%); and shortleaf pine damage was the least damaged of all species (2.9%). In full exclusion plots, herbivory was generally low: white oak was damaged most frequently (14.8%); black locust damage was lower (3.8%); and shortleaf pine herbivory in full exclusion plots was nearly absent (0.2%).

Table 3. First-year mammal herbivory incidence (%; Mean ± SE) among tree species and exclusion treatments on reclaimed mined lands in southeastern Kentucky. Means with differing letters indicate significant differences among exclusion treatments within a species, and means with different symbols indicate significant differences among species within an exclusion treatment, as determined via Type III ANOVA and subsequent Tukey-corrected pairwise comparisons at a 0.05 significance level.

<table>
<thead>
<tr>
<th>Species</th>
<th>No Exclusion Mean ± SE</th>
<th>Protector Mean ± SE</th>
<th>Full Exclusion Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Locust</td>
<td>85.1a ± 2.7</td>
<td>73.8b ± 6.7</td>
<td>3.8c ± 1.2</td>
</tr>
<tr>
<td>Shortleaf Pine</td>
<td>34.1a † ± 7.0</td>
<td>2.9b ‡ ± 1.5</td>
<td>0.2c ‡ ± 0.2</td>
</tr>
<tr>
<td>White Oak</td>
<td>72.6a † ± 7.2</td>
<td>51.1b † ± 3.9</td>
<td>14.8c * ± 3.2</td>
</tr>
</tbody>
</table>

The cumulative herbivory rate for all seedlings in the study was 33.2%, of which cervids were responsible for 95.8%. Of all black locusts damaged in each of the exclusion treatments, cervid herbivory accounted for at least 93%, with minor contributions by rabbits (0.4–6.7%) and small mammals (1.7%; Table 4). Cervids mediated 74.7% and 50% of shortleaf pine damage in no exclusion treatments and protector treatments, respectively; rabbits were culpable in the damage of the remaining shortleaf pines in these treatments (25.3% and 50%, respectively). Rabbit herbivory comprised all damage to shortleaf pines in full exclusion treatments. Similar to black locust, cervids were responsible for at least 91% of all white oak herbivory in each exclusion treatment; rabbit contribution to white oak damage was also similar to that of black locust (1.8–6.7%). Small mammal herbivory was highest.
on white oaks in no exclusion treatments (3.7%). A single uprooted white oak (0.6%) in no exclusion treatments was attributable to herbivory by domestic animals (i.e., horse).

Table 4. Relative herbivore contribution (%) to herbivory incidence by tree species and exclusion treatment on reclaimed mined land in southeastern KY. Damage of a seedling by multiple taxa results in total contributions greater than 100%.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cervid</th>
<th>Rabbit</th>
<th>Small Mammal</th>
<th>Domestic Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Black locust</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Exclusion</td>
<td>98.8</td>
<td>1.3</td>
<td>1.7</td>
<td>-</td>
</tr>
<tr>
<td>Protector</td>
<td>99.6</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Full Exclusion</td>
<td>93.3</td>
<td>6.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Shortleaf pine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Exclusion</td>
<td>74.7</td>
<td>25.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protector</td>
<td>50.0</td>
<td>50.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Full Exclusion</td>
<td>-</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>White oak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Exclusion</td>
<td>97.5</td>
<td>1.8</td>
<td>3.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Protector</td>
<td>97.9</td>
<td>2.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Full Exclusion</td>
<td>91.7</td>
<td>6.7</td>
<td>1.7</td>
<td>-</td>
</tr>
</tbody>
</table>

4. Discussion

Tree species and herbivore exclusion treatment significantly influenced survival, height growth, and herbivory damage. Black locust and white oak survival increased with exclusion presence; however, there was no difference in survival between protector or full exclusion treatments. Conversely, while shortleaf pine survival was low across all treatments, survival was similar in no exclusion and protector treatments but significantly lower in full exclusion treatments. Black locust typically sustains moderate to high survival (53–100%) on mined sites in the first three to five years after planting [28,39], attributing to its favorability for mine reforestation. White oak survival in this study (68.2–80.5%) was also similar to that found by Emerson et al. (2009) [39] when planted within weathered gray and unweathered brown sandstone mine spoils (70–80%) and by Bell et al. (2017) [40] when planted in a mixed pine-hardwoods polyculture (50–80%). Shortleaf pine survivals observed in this study were at the lower extent of shortleaf pine survivals found by Bell et al. (2017; 29–58%) [40].

Herbivore exclusion has effectively increased the survival of natural regeneration and reforestation plantings in many systems, often due to a reduction in herbivory incidence and severity [41–43]. On reclaimed mined lands in eastern KY, tree shelters successfully increased the initial survival of direct-seeded chestnuts (*Castanea* spp.) [44]. Fencing is generally successful at increasing seedling survival through large-ungulate exclusion [45–48]; however, its use in Appalachian surface mine reforestation appears limited, as the present study is, to our knowledge, the first to evaluate the effectiveness of exclusion at reducing herbivore damage in this region. In this study, shortleaf pine survival in full exclusion treatments was significantly lower than that in other treatments, which is possibly due to higher levels of competing vegetation in full exclusion plots and lower initial heights of pine seedlings compared with black locust and white oak. Reduced survival rates as a result of fencing have been shown for black cherry (*Prunus serotina* Ehrh.) on reclaimed mines in Indiana [49].

Positive height growth was observed for all species in this study; however, black locust growth was significantly higher than that of shortleaf pine and white oak. Black locust is a pioneer species that naturally colonizes disturbed areas and can persist in environmentally harsh conditions due to its rapid initial growth rates [50,51] and ability to form symbiotic relationships with N\(_2\)-fixing bacteria [52], justifying its use for the reforestation of mined lands, landfills, and degraded areas that are often nutrient-depleted [39,53]. First-year growth of black locust in this study was much greater than that of black locusts planted on adjacent reclaimed mined sites in eastern KY (9.4 cm) and was even higher than that of fertilized black locusts (20.4 cm) [54]. Mean white oak growth in this study
(8.6 cm) was somewhat higher than that of white oaks planted in pine-hardwood polyculture in eastern KY (5.6 cm); height growth of northern red oak (Quercus rubra L.) and chestnut oak (Quercus montana Willd.) was also lower than that of white oak found in the present study [40]. Mean tree heights three years post-planting reported by Showalter et al. (2007) [55] in response to spoil type in Virginia appear to indicate growth rates similar to those in this study. Mean shortleaf pine heights reported by Bell et al. (2017; 10.5 cm) [40] were comparable to mean heights in this study. Similar first-year growth rates for shortleaf pine were also found by Kabrick et al. (2015) [56] for underplanted pines in the Missouri Ozark Highlands, indicating that shortleaf pine growth on reclaimed surface mines may approximate that of one-year-old pines regenerating under a closed-canopy forest.

Exclusion treatment significantly affected seedling height growth. Protector treatments cultivated the highest growth rates. Seedling protectors (or tree shelters/tubes) have increased the tree growth of a variety of deciduous and coniferous species [44,57–59], not only from a decreased impact of herbivory, but also in their effect on growing conditions. Protector construction can either improve or inhibit seedling growth rates [58,60–62]. Microclimate variables affecting growth rate (e.g., relative humidity, radiation absorption, CO₂ concentrations) vary with and within protector types [58,61]. Andrews et al. (2010) [62] demonstrated elevated hardwood growth rates in riparian forest corridors due to tree shelter use, attributed to woody debris retention around the protector and physical protection against flooding. Protectors selected for this study were manufactured of plastic interwoven in a diamond pattern with 2–3 cm openings. The protector’s construction accommodated air flow between the atmosphere and the interior of the protector and limited shading effects to seedlings; therefore, the increased growth rate is, at best, marginally attributable to improved microclimate. Since soil analyses yielded no significant difference among treatments for the selected parameters, height growth responses of protector treatments are likely more associated with increased stem elongation as a result of protector presence and with competing vegetation dynamics. Growth in full exclusion treatments (also employing protectors) was significantly lower than that in protector treatments: competing vegetation was observed to be taller in full exclusion treatments compared with non-fenced treatments, potentially from decreased herbivory prevalence compared with outside of exclosures. Therefore, protector presence and reduced competing vegetation are likely responsible for the improved growth rate fostered by protectors.

Herbivory incidence in this study was driven by an interaction between tree species and exclusion treatment. All species in this study responded similarly to exclusion: herbivory was greatest in plots with no exclusion; protectors significantly lowered herbivory, but full exclusion treatments vastly reduced herbivory. Cervids were responsible for nearly 96% of all herbivory. Therefore, fencing was effective at limiting damage, but did not fully prohibit plot access to cervids. While no animals were observed within any fenced plots, beds and trails were observed within the plots, and deer and elk tracks, scat, and hair were found on fence perimeters on multiple occasions. Regardless, herbivory incidence was reduced as a result of fencing. Protectors also effectively reduced herbivory compared with no exclusion treatments. Although cervids damaged seedlings within protector treatments, the treatment effect is speculatively driven, in part, by relative seedling height: certain seedlings did not grow beyond the top of the protector in the first growing-season; thus, they were not available for browsing, demonstrating that smaller seedlings are protected against herbivory while gaining root mass and leaf area, which will aid in resilience to herbivory once the seedling has grown above the top of the protector.

In no exclusion treatments, a definitive herbivory preference was observed for black locust (85.1%) and white oak (72.6%). On adjacent mined lands in eastern Kentucky, black locusts in control plots sustained two-year browse rates of 76%, but as high as 91% black locust browse was observed after soil fertilization [28]. Due to elevated shoot N levels [28], black locust is foraged preferentially by ungulates [63]. While white oak was preferred significantly less than black locust, herbivory of this species was, nonetheless, considerable. On reclaimed mined land in Indiana, first-year deer browse rates of white oak in unexcluded plots was approximately 90% [49]. Mixed hardwoods, in general,
appear to be heavily browsed during the first year: black cherry (90%), bur oak (*Quercus macrophylla* Michx.; 89%), and northern red oak (84%) were heavily damaged by deer in Indiana [49]. Likewise, Skousen et al. (2009) [64] reported “heavy browse” of white ash (*Fraxinus americana* L.) on mines in West Virginia. Negative height growth of chestnut oak and northern red oak was attributed by Bell et al. (2017) [40] to deer and elk browse; however, American sycamore (*Platanus occidentalis* L.) was relatively undamaged (<3%) in the second growing-season on mined land in eastern Kentucky [28]. Additionally, pines seem to be less preferred by herbivores compared with hardwoods. In this study, the shortleaf pine herbivory rate in no exclusion treatments was 34%. Cumulative browse of unexcluded underplanted eastern white pine (*Pinus strobus* L.) in northern forests was less than 43% [59]. Tree species selection for planting mixtures is an active area of research and one that will greatly benefit from mine reclamation efforts.

This study has revealed that herbivory on reclaimed Appalachian mined land is extensive and that techniques for control require further consideration. Although herbivory by rabbits, small mammals, and domestic animals was documented, cervids accounted for nearly all first-year herbivory damage. Deer populations have increased markedly across the eastern U.S. over the previous decades. Similarly, elk reintroduction has become a nearly widespread management goal of state wildlife agencies across Appalachia, with successful population establishment in five states (Kentucky, North Carolina, Pennsylvania, Tennessee, and West Virginia) [65]. Concomitant with deer population explosion, elk expansion will intensify herbivory pressure, especially of reforestation plantings on reclaimed surface mines, where most elk releases in Kentucky and neighboring states have occurred. Horse populations on reclaimed mines will likely continue to increase; although the results of this study indicate that horse impacts are minimal, this source of herbivory should continue to be monitored on a local scale, specifically in areas with high populations.

5. Conclusions

Seedling protectors successfully lowered herbivory incidence during the first growing-season following planting; however, full exclusion drastically reduced herbivory, yet fencing was not effective at fully excluding cervids. Exclusion treatments also generally increased seedling survival and height growth. While exclusion has been found to be effective at limiting herbivory damage, these treatments may likely prove economically or logistically unfeasible in some circumstances. The cost of fencing (material and labor) for this study was approximately $21,220 per ha, and protector material and installation costs were approximately $0.60 per seedling ($1,500 per ha at study planting densities). Although fencing effectively negated herbivory damage and increased first-year survival rates compared with no exclusion plots, managers must decide if the large initial investment in fencing is offset by the future value of the forest resources. Protectors are a more economical method of reducing herbivory and promoting height growth; however, once seedlings grow beyond the top the protector, cervids damage the upper shoots, which will, ultimately, hinder height growth and create poor growth form. Therefore, tree species less preferred by herbivores (i.e., cervids) should be identified for inclusion in planting mixes to reduce herbivory impacts to forest recruitment. Black locust and white oak were found to be highly preferred by cervids, but shortleaf pine was selected less frequently. These results indicate that hardwood regeneration on mined lands will likely prove difficult with current and projected future cervid population levels. Restoration of pine forests on Appalachian surface mines may be more successful given lower herbivory rates; however, low survival rates may preclude this effort. Follow-up seedling assessments in three to five years will provide additional results on herbivory impacts during the years when seedlings are most susceptible to herbivory damage.
Acknowledgments: Support for purchasing and planting of seedlings was provided by the KY Sustainable Forestry Initiative Sustainable Implementation Committee. Partial funding was also provided by the National Fish and Wildlife Foundation, TreeCycler, Arbor Day Foundation, University of Kentucky College of Agriculture, Food, and Environment, and the University of Kentucky Appalachian Center. We thank all of the UK Robinson Center for Appalachian Resource Sustainability (RCARS) and Robinson Forest employees who helped with fence construction. We are grateful to J. Frederick, M. Anderson, D. Thomas, Z. Grigsby, W. Dixon, A. Davis, and W. Leuenberger for assistance with field measurements.

Author Contributions: All authors obtained funding, designed research methods, and wrote (or substantially edited) the manuscript. Z.J.H. collected and analyzed data.

Conflicts of Interest: The authors declare no conflict of interest.

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