EVALUATING SPOIL AMENDMENT USE AND MYCORRHIZAL INOCULATION ON REFORESTATION SUCCESS IN THE EASTERN AND WESTERN KENTUCKY COALFIELDS[1]

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Abstract: A factorial experiment using inoculated vs. non-inoculated forest species in non-fertilized, fertilized only, amended (organic mulch) only, and fertilized + amended plots was established in 2004 on two mined lands in the Eastern (EKY) and Western (WKY) Kentucky coalfields. Fifty-four 15 x 15 meter plots were delineated on each site. Half of the plots received an application of 40 tons per acre of a wood chip/manure compost mixture. All plots were subsequently ripped to a depth of approximately 2-meters using a dozer. Two tree species; Loblolly Pine (LP) (*Pinus taeda*) and Northern Red Oak (NRO) *(Quercus rubra*), were planted in the plots. Each plot received 100 seedlings spaced at 1.5 meter centers. The mycorrhizal trees were inoculated with *Pisolithus tinctorius* (Pt) and *Scleroderma cepa* (Sc) in the nursery beds. Additional spores were applied in the field after they were transplanted. The non-mycorrihizal plots received an application of fungicide on an annual basis (Bayleton; 1 kg per plot) to suppress natural inoculation. Fertilized plots received 150 pounds of 20-20-20 fertilizer per acre (168 kg per ha) on an annual basis. Each treatment was examined in triplicate for each species. Ripping of the sites reduced in-situ bulk density at both locations. Application and incorporation of the compost resulted in a further decrease in bulk density both at the surface and through the entire 30.5-cm depth examined. Although the effect was similar between the two sites, the WKY site exhibited a higher final bulk density than that observed in EKY. After four years, growth and survival of planted seedlings differed with respect to species, site and treatments. LP exhibited almost a doubling in growth at the EKY mine as compared to the WKY mine. Amendments had no effect on LP growth at the WKY site. All amendments had a positive effect on LP growth over that of the control in the EKY site. Use of compost and mycorrhizal fungi yielded the highest survival for LP at both sites. NRO growth was very low compared to LP and did not differ drastically between the two sites. Treatment effects were observed on both sites and results varied. Compost and fertilizer without mycorrhizae exhibited the highest NRO survival at both sites. Fertilizer addition improved NRO survival at the EKY mine and the use of compost without fertilizer appeared to increase mortality. The use of compost with mycorrhizae may have also had an inhibitory effect on NRO survival in WKY, or fungicide application may have provided a positive effect at this site. Herbaceous biomass was much higher on the EKY mine, but no correlations between herbaceous biomass and tree growth or survival were observed for either site.

Additional Key Words: forest reclamation approach, soil amendments, topsoil replacement.

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Introduction

Prior to SMCRA, reforestation was the reclamation technique of choice in the east, but a steady decline in the amount, diversity, and productivity of forestland has been observed since (Burger 1999). Attempts to reforest post-law sites often resulted in high seedling mortality, slow growth, and poor production typically due to highly compacted soils with limiting chemical characteristics and intense competition from ground cover (Ashby, 2006).

SMCRA necessitates that reclaimed mined land be stable and returned to its "approximate original contour" (AOC) (United States Congress 1977). To achieve this, bull dozers are used to repeatedly compact the residual spoil material. Consequently, the reclaimed land is so compact that it prohibits root development and tree establishment. Specifically, compacted soil increases bulk density and soil resistance to mechanical penetration, and reduces hydraulic conductivity, as well as water, air and nutrient flow in the soil (Barnhisel 1988). SMCRA also requires that mined land be reclaimed to a point where it may "support the uses which it was capable of supporting prior to any mining, or higher or better uses" (United States Congress 1977). After the passage of SMCRA, coal operators more often chose hayland or wildlife habitat instead of forest for revegetation due to bond release expediency and satisfaction of regulatory requirements concerning sedimentation and erosion control. Burger (1999) reported that 95% of previously forested mined lands are reclaimed to a hayland or wildlife habitat post mining land use, and are abandoned to eventually revert to forests through natural succession which can take upwards of 120 years for eastern deciduous forests.

Until recently, many landowners and mining companies saw tree planting on post-law mined lands as a waste of time because of the poor results of early trials and due to misconceptions (Ashby 1998). Recent findings, however, have produced promising results and a resurgence in reforestation has been observed in the eastern US coal field (Angel et al. 2007; Michels et al. 2007; Angel et al. 2006; Burger and Zipper 2002; Conrad et al. 2002; Rodrigue 2002; Graves et al. 2000; Torbert et al. 2000; Ashby 1999; Burger and Torbert 1992). Not only has success been observed on sites that are reclaimed using low compaction techniques, but success is also being observed on previously compacted sites that were loosened by mechanical means (Sweigard et al. 2007; Michels et al. 2006). Successful reforestation techniques for surface mined lands continue to be studied, developed, and streamlined, but questions remain. Given that there are potentially hundreds of thousands of acres of reclaimed and abandoned grasslands that could be reforested (Zipper et al. 2007), efforts to determine whether specific surface and sub-surface treatments improve growth and survival of forest species on these areas are needed.

Detailed studies to address specific questions pertaining to reforestation success were initiated with the establishment and manipulation of plots to examine the influence of mycorrhizae, spoil chemical and mineralogical properties, and use of amendments on mineland forest establishment. Based upon earlier studies, mycorrhizal fungi have been shown to play a major role in the survival, nutrient uptake and biomass development (above and below ground) of seedlings planted on mined sites and abandoned industrial areas (Barton et al. 2005; Marx et al. 2002, and references therein; Kumar and Upadhyay, 1999). Other studies have shown the benefits of organic composts for minimizing compaction, enhancing fertility and retaining moisture in spoils and degraded areas (Angel et al., 2006; Barton et al., 2005; Ringe et al. 1989; Evangelou, 1981). However, the success of these methods in compacted spoils, reclaimed as hayland pasture, that were loosened by ripping has not been established. As such, a factorial experiment using inoculated vs. non-inoculated species in non-fertilized, fertilized only, amended (organic mulch) only, and fertilized + amended plots was established on ripped surface mined lands in 2004.

Methods

Research plots were established on two reclaimed mine sites in the Eastern (Laurel Fork, Knott County) and Western (Nelson Creek, Muhlenberg County) Kentucky coal fields. Fifty-four 15 x 15 meter plots were delineated on each site. Half of the plots received an application of 40 tons per acre of a wood chip/manure compost mixture. Afterwards, each site was ripped to a depth of approximately 2-meters using a D-9 dozer (Figs. 1 and 2). Seedlings used for this study were grown at the Kentucky Division of Forestry Nursery, Morgan County nursery. The mycorrhizal trees were initially inoculated with *Pisolithus tinctorius* (Pt) and *Scleroderma cepa* (Sc) in the nursery beds. The mycorrhizal plots received additional spore application in the field after they were transplanted. Per the manufacturer’s instructions, approximately 10 g of MycorTree® ecotomycorrhizal spores were mixed with 5 L of water, and each seedling was treated with approximately 100 mL of this solution. The spore application was performed to potentially enhance the inoculation index of the planted seedlings. The non-mycorrihizal plots received an annual application of fungicide (Bayleton; 1 kg per plot) to suppress natural inoculation. Fertilizer plots received 150 pounds of 20-20-20 fertilizer per acre (168 kg per ha) on an annual basis for the first three years of the study. Two tree species; *Pinus taeda* (Loblolly Pine, LP) and *Quercus rubra* (Northern Red Oak, NRO), were planted in the plots. Each plot received 100 seedlings spaced at 1.5 m centers. Each treatment was examined in triplicate for each species. At 100 trees per plot x 3 plots per treatment x 8 treatments x 2 species, 4,800 seedlings were planted per site. Half of the seedlings from each plot were tagged and repeatedly measured for height and diameter and survival statistics. Growth was calculated as the Year 4 (2007) height minus initial height (2004). Tree volume was determined on the Year 4 seedlings by the equation: $v = d^2 * h$, where v = volume, d = diameter and h = height.

Soils

Soils (spoils) were sampled immediately after ripping and again each summer (2004-2007). Samples were collected at depths of 0-10 cm and 20-40 cm and analyzed for pH, EC (as soluble salts), texture, cation exchange capacity, base saturation, soluble P, exchangeable nitrate and ammonium, total C, and total N. All analyses were performed using procedures outlined by the Soil Survey Staff (NRCS, 1996). Bulk densities of soils at 5 cm, 15 cm and 30.5 cm were determined in-situ using a Nuclear Moisture Density Gauge 3440 (Troxler Electronic Laboratories, Inc. - Research Triangle Park, NC) during the first two years of the study.

Figure 1. Compost amended site, Knott Co. KY.

Figure 2. Reclaimed pasture was ripped to alleviate compaction in preparation for tree planting in Knott Co. KY.

Herbaceous Biomass

A herbaceous biomass survey was performed in years 2, 3 and 4 in each plot using two randomly located (1.0 x 1.0m) subplots. All herbaceous vegetation within each subplot was cut at ground level, collected, oven-dried at 60^{o}C and weighed for biomass.

Statistical Analysis

After four growing seasons, survival data of the seedlings were analyzed with repeated-measures logistic regression models (PROC GENMOD). The models included all main-effects and two-way interactions, with survival as the dependent variable, and treatments (mycorrhizae/compost/fertilizer/control) as the independent variables. Probabilities of seedling survival were calculated by back transformation of the least-squares mean (LSM) from the logistical models (e^{LSM}) / (1 + e^{LSM}).

Seedling growth was estimated by subtracting the initial height from the height at the end of the fourth growing season. Height measurements for the seedlings were analyzed with linear regression models (PROC MIXED). The models included all main-effects and two-way interactions, with seedling height as the dependent variable and amendment treatments as the independent variables. Differences were considered to be statistical significant when $p < 0.05$. All statistical analyses were performed using SAS (SAS 1999).

ANOVA and means comparisons (Tukey-Kramer HSD) were used to detect significant differences between herbaceous biomass in the plots. Statistical significance was established where $p < 0.05$.

Results and Discussion

Compaction Alleviation

Both sites were ripped prior to planting to alleviate compaction created by initially reclaiming the sites as pasture and to incorporate compost where applied. The ripping provided good results at the EKY mine by reducing in-situ bulk density in the upper 5-cm of spoil to 1.23 g $cm^{3\ -1}$ (Table 1). Application and incorporation of the compost resulted in a further decrease in bulk density both at the surface and through the entire 30.5-cm depth examined. Incorporation of the compost was expected not only to reduce compaction but also to allow for better root penetration, gas exchange and water infiltration (Bledsoe et al. 1992).

Table 1. Mean bulk density at 5, 15 and 30.5 cm depths in spoil (control) and compost amended spoil (compost) at the Laurel Fork mine in Knott Co. KY (EKY).

Treatment	Year	Bulk Density (g $cm^{3\ -1}$)		
		5 cm	***15 cm***	***30.5 cm***
Control	**2004**	1.23	1.48	1.65
Control	**2005**	1.21	1.49	1.66
Compost	**2004**	1.02	1.32	1.54
Compost	**2005**	0.95	1.33	1.55

Ripping also provided good results at the WKY mine with a reduction in bulk density in the upper 5-cm of spoil to 1.36 g $cm^{3\ -1}$ (Table 2). As with the Laurel Fork site, application and incorporation of the compost resulted in a further decrease in bulk density both at the surface and through the entire 30.5-cm depth examined. Although the effect was similar between the two sites, the WKY site exhibited a somewhat higher final bulk density than that observed at the EKY site in the 5 and 15-cm depths. Interestingly, both sites exhibited lower bulk densities than those reported for loose-graded (uncompacted) sites on other eastern Kentucky reforestation projects that showed high seedling survival rates (Angel et al. 2007; Michels et al. 2007; Angel et al. 2006).

Table 2. Bulk density at 5, 15 and 30.5 cm depths in spoil (control) and compost amended spoil (compost) at the Nelson Creek mine in Muhlenberg Co. KY (WKY).

Treatment	Year	Bulk Density (g $cm^{3\ -1}$)		
		5 cm	***15 cm***	***30.5 cm***
Control	**2004**	1.36	1.51	1.65
Control	**2005**	1.31	1.49	1.65
Compost	**2004**	1.12	1.39	1.56
Compost	**2005**	1.15	1.37	1.52

Seedling Response

Prior to planting, twenty-five seedlings of each species were collected for an assessment of growth characteristics and mycorrhizal inoculation index. Average height was 39.7 and 18.4 cm for the NRO and LP, respectively. The NRO exhibited an average diameter of 5.1 mm, while the LP had an average diameter of 3.4 mm. The inoculation index for both species was low as exhibited in Table 3. As such, mycorrhizal plots received additional inoculation in the field to further enhance these numbers. Non-mycorrhizal plots received an annual application of the fungicide Bayleton® to suppress natural inoculation.

Table 3. Mycorrhizal inoculation index of seedlings prior to planting.

Species	Inoculation Index (%)†		
	Pt	***Sc***	***Tt***
N. Red Oak	18	3	0
Loblolly Pine	26	9	1

†*Pt = Pisolithus tinctorius; Sc = Scleroderma cepa; Tt= Thelephora terrestris*

Treatment effects on seedling vigor were noticeable very soon after transplanting occurred (Fig. 3). After four years, growth and survival of planted seedlings differed with respect to species, site and treatments. Loblolly pine exhibited an increase of nearly 1.5 times in height growth and a 7.3 fold increase in volume index in EKY as compared to the WKY site (Table 4). Amendments had no statistically significant effect on LP growth at the WKY mine with rates between 86 and 94 cm. All treatments exhibited significant increases in LP growth over that of the control at the EKY site (Fig. 4). The use of mycorrhizae improved LP growth in the absence of compost in EKY. When compost was applied in EKY, neither the mycorrhizae nor the fertilizer treatments statistically improved the growth of LP.

Figure 3**.** One-year-old NRO seedling planted in non-amended (a) and compost amended (b) ripped spoil at the Muhlenberg County (WKY) site.

Table 4. Seedling growth in height and volume index for 4-yr loblolly pine and northern red oak growing under differing spoil amendment treatments at the EKY and WKY mines in Knott and Muhlenberg Counties, KY, respectively.

Treatment*	**EKY**		**WKY**	
	Growth (cm)	***Volume (cm^3)***	***Growth (cm)***	***Volume (cm^3)***
LCFM	152.8 (AB)‡	2646	79.5 (A)	254
LCFN	157.1 (A)	3063	86.0 (A)	422
LCM	163.2 (A)	3628	87.1 (A)	372
LCN	146.4 (AB)	2510	93.6 (A)	541
LFM	154.2 (A)	3060	94.1 (A)	499
LFN	133.7 (B)	1975	93.5 (A)	331
LM	133.0 (B)	1993	87.3 (A)	243
LN (control)	110.6 (C)	1387	77.8 (A)	213
Mean	*127.1*	*2532*	*87.3*	*348*
NROCFM	15.7 (AB)	68	11.2 (BC)	45
NROCFN	13.8 (AB)	47	22.3 (AB)	75
NROCM	11.8 (AB)	52	-4.5 (C)	15
NROCN	7.8 (B)	27	12.5 (BC)	36
NROFM	15.1 (AB)	61	26.3 (AB)	37
NROFN	22.2 (A)	117	28.3 (AB)	50
NROM	14.0 (AB)	81	43.3 (A)	94
NRON (control)	5.5 (B)	41	19.7 (B)	26
Mean	*13.2*	*61.7*	*19.9*	*47.3*

*L = loblolly pine; NRO = northern red oak; C = compost; F = fertilizer; M = mycorhizal; N = no mycorhizae.

‡Means for individual species and location followed by the same letter are not significantly different (p = 0.05)

Figure 4. Four-year-old LP seedlings planted in non-amended (a) and compost + mycorrhizal amended (b) ripped spoil at the Knott County (EKY) site.

Survival of LP was higher at EKY (mean = 77%) than WKY (mean = 44%) for all treatments examined (Table 5). The lowest survival for LP at EKY was 60% (LCN), which was higher than all treatments at WKY except LCM. Unlike the growth results, there are no apparent trends for LP survival at either site that can be attributed to the amendments or inoculation. The compost + mycorrhizae treatment; however, did exhibit the highest LP survival rate at both sites.

Northern red oak growth was poor compared to LP and did not differ significantly between the two sites (Table 4). Treatment effects were observed on both sites and results varied. As with LP, survival of red oak was higher at EKY than WKY for all treatments except NROCN (Table 5). Compost and fertilizer without mycorrhizae exhibited the highest NRO survival at both sites. Fertilizer addition improved NRO survival at the EKY mine. Increased mortality was also observed at EKY when compost was applied with no additional fertilizer. The use of compost with mycorrhizae may have also had an inhibitory effect on NRO survival in WKY. Highest red oak survival was observed in the NROCFN treatment for both sites.

Table 5. Seedling survival for 4-yr loblolly pine and northern red oak and herbaceous biomass on plots with differing spoil amendment treatments at the EKY and WKY mines in Knott and Muhlenberg Counties, KY, respectively.

Treatment*	**EKY**		**WKY**	
	Survival (%)	***Herb Biomass ($g\ m^{2-1}$)***	***Survival (%)***	***Herb Biomass ($g\ m^{2-1}$)***
LCFM	69 (BC)‡	391	48 (AB)	85
LCFN	75 (B)	324	44 (B)	128
LCM	92 (A)	268	61 (A)	139
LCN	60 (C)	224	52 (AB)	96
LFM	85 (AB)	352	27 (C)	248
LFN	83 (AB)	215	40 (B)	162
LM	84 (AB)	222	35 (B)	73
LN (control)	73 (BC)	305	44 (B)	158
Mean	*77*	*287*	*44*	*136*
NROCFM	60 (BC)	505	25 (D)	110
NROCFN	79 (A)	243	60 (A)	102
NROCM	43 (D)	282	21 (D)	114
NROCN	31 (D)	427	41 (BC)	141
NROFM	76 (AB)	392	48 (AB)	177
NROFN	68 (ABC)	323	43 (BC)	233
NROM	69 (ABC)	467	32 (CD)	185
NRON (control)	57 (CD)	210	44 (BC)	168
Mean	*60*	*356*	*39*	*170*

*L = loblolly pine; NRO = northern red oak; C = compost; F = fertilizer; M = mycorhizal; N = no mycorhizae.
‡Means for individual species and location followed by the same letter are not significantly different ($\alpha = 0.05$)

In general, the use of mycorrhizal fungi, irrespective of the other amendments, exhibited significantly higher LP volume indices (≈ 600 cm^3 increase) and survival (10% increase) over that of the non-mycorrhizal plots at EKY. Similar responses have been noted by Marx et al. (2002). Mycorrhizal plots also showed some enhancement of survival and growth of NRO in EKY, but significant increases in growth or survival attributable to the mycorrhizae were not observed for either LP or NRO in WKY. In fact, most of the WKY NRO mycorrhizal plots performed worse than their paired non-mycorrhizal plots (e.g. NROCFM vs. NROCFN), which may suggest that the fungicide provided a benefit to the seedlings on those plots. Specific pathological tests were not performed on affected trees at this site, but plant pathogens such as *Phytophthora* and *Rhizoctoniz* spp. were detected on other ripped reforestation sites we planted and are known to affect NRO. These pathogens, and other oomycetes detected on affected plants growing in previously reclaimed pastures that had been ripped and reforested, are commonly associated with wet soil conditions.

Survival and growth differences between the sites were initially suspected to be caused by competition from aggressive herbaceous species found within the plots. Herbaceous biomass content measured from clippings taken at each site in years 2, 3 and 4, however, revealed that competition may not have been a problem. In general, biomass was much higher on the EKY site (which exhibited higher survival and growth) as compared to WKY (Table 5). Within the sites there was no significant correlation between survival by treatment and biomass. Nutrient content of the soils within the plots, as expressed by Total C, Total N, exchangeable nitrate and ammonium contents, varied by treatment and between plots but also did not correlate with survival and growth trends. The decreased bulk density at EKY, compared to WKY, likely had some positive effect on seedling survival (Tables 1 and 2), but significant relationships were not found. Even though ripping appeared to alleviate the surface compaction, textural differences in spoils between the sites may have influenced drainage below the plow layer. A topsoil variance was used at the EKY site and spoils were derived from sandstone with textures at the 20-40 cm depth of loam to sandy loam. Stockpiled soil was replaced at the surface of the WKY site and textures at the 20-40 cm depth were clay loam to clay. The increased clay content may have influenced drainage at WKY resulting in increased mortality and lower growth rates as compared to EKY. Further assessments of other spoil physicochemical properties and their influence on hydraulic conductivity are planned to provide additional information on site differences. Pathological analysis of affected plants from these sites will also be evaluated in the future.

Conclusions

Use of spoil amendments to improve reforestation success on ripped surface mine lands that were previously reclaimed as pasture was evaluated. Forest productivity varied across the state and between different mine sites. Seedling success was found to be species specific and, in some cases, strongly influenced by amendment. Although the stands are young (4-yr old), some general inferences to success and productivity potential may be ascertained. Soil amendments in the form of mycorrhizal fungi, compost and fertilizer appeared to improve productivity of LP in plots located in EKY, but not in plots located in WKY. For NRO; however, productivity and survival varied widely. Use of inorganic fertilizers, with or without compost and mycorrhizae, resulted in increased survival of NRO in EKY. At the WKY mine, NRO survival appeared to benefit from the use of a fungicide which was applied to suppress mycorrhizal infection. Even though ripping and compost application appeared to alleviate compaction from the previous reclamation efforts at these sites, spoil type differences with respect to physical make-up and hydrologic properties may have ultimately influenced reforestation success on these sites.

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