ESTABLISHMENT OF NATIVE PLANT SPECIES FROM FOREST TOPSOIL SEEDBANKS ON A BORROW AREA IN KENTUKCY[1]

by

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Abstract. The seed banks in forest topsoils were used to introduce native species to an unreclaimed, xeric borrow area in eastern Kentucky. The results of using eight treatments are reported: (1) 1 cm topsoil + mulch, (2) 1 cm topsoil tilled into the substrate + mulch, (3) 2 cm topsoil + mulch, (4) 1 cm topsoil without mulch, (5) 4 cm topsoil in strips covering 25% of the area + mulch, (6) 1 cm topsoil + a grass-legume reclamation mix + mulch, (7) the reclamation mix + mulch without topsoil, and (8) mulch without topsoil or reclamation mix. The seed bank produced 90 species from 34 families including seven tree species, seven shrubs, 16 graminoids, and 50 forbs. Forest topsoil use introduced 57 native or naturalized species during the first growing season and 82 species were present during the second growing season. Average ground cover of native species totaled only about 5% after ten weeks of the first growing season. Wheat and timothy seed contained in the hay mulch increased mean ground cover to over 70%. The reclamation mix produced 96% cover during the first year and it completely suppressed establishment of native species during the first two years. Different methods of topsoil use without the reclamation mix did not significantly affect the number of native species established in the first and second growing seasons, cover in the first growing season, the number of species increase in the second year, or the number of established trees and shrubs.

Additional key words: Reclamation, revegetation

Introduction

Drastic land disturbances such as surface mines, road banks, quarries, and construction projects with fills or borrow areas produce significant areas requiring revegetation and reclamation. Techniques for simple revegetation of these areas are well developed, but exotic and monotonous, highly competitive plant communities are the frequent result. Also, establishment and succession of desirable communities of native species may be slowed by current revegetation practices (Brenner 1984). Communities of native species can be established with commercially available seed and plantint stock, but such materials tend to be expensive, and propagules of many native species are often not available.

Topsoil seed banks have been used effectively to introduce native species on reclaimed mined lands in the western United States (Beauchamp et al. 1975, Howard and Samuel 1979, Iverson and Wali 1982). However, the amount of cover produced by the seed bank species has been less than that obtained with exotic reclamation species. DePuit (1984) has synthesized some potentially successful strategies for enhancing vegetation and species diversity on reclaimed mined lands using topsoil seed banks.

Use of topsoil seed banks to introduce native species to reclaimed land has been successful in Australia. Tacey (1979) used the top 5 cm of topsoil to introduce native species of jarrah (Eucalyptus marginata) forest to mined lands in Western Australia. Dominance-diversity curves in reestablished vegetation approached the log-normality distribution found in more diverse communities after four years (Tacey and Glossop 1980). Topsoil seed banks have been used to reestablish many high dune forest and coastal wetland species after surface mining on the Australian east coast (Brooks 1988).

In the United Kingdom, chopped turf from old mined sites has been used to introduce native

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species to newly mined sites. A 30 cm layer of topsoil was also successful in native species introduction, but a "few millimeters thick" and "small molehill" amounts were not effective (Wathern and Gilbert 1978, Gilbert and Wathern 1980).

Farmer et al. (1982) and Wade (1986) found that seed banks in eastern United States deciduous forests produced many native species in pioneer communities with high biomass and cover when forest topsoils were spread 2.5 to 5.0 cm thick over mine spoils in 1.3 m^2 microplots. Moreover, the growing conditions in these last two studies were optimal because of supplemental irrigation.

Quality of the seed bank is important for species introductions. Quality can be influenced by source soil types, vegetation, disturbance history, aspect, and season of collection. A soil may contain several types of seed banks that range from transient to persistent based on seed-dormancy characteristics of its component species. Different types of seed banks may be able to exploit certain types of disturbances at only certain times of the year (Grime 1981).

Many reclamation/revegetation projects occur on sites where stressful conditions of high temperatures, low fertility, and low available water potential exist. Large quantities of topsoils for large-scale projects can be difficult to obtain without disturbance to other areas on an unacceptable scale. The borrow area of Cowbell Hollow provided an opportunity to investigate: (1) what methods of application of minimal amounts of forest topsoil would establish pioneer communities from the contained seed bank, (2) whether exotic reclamation ground cover species would inhibit the invasion and establishment of desired native successional species, and (3) which species could be established after two growing seasons.

Materials and Methods

The study area is a 7.0 ha borrow area within the Red Lick Watershed Control Basin below Cowbell Hollow Reservoir in the Berea College Forest, Madison County, Kentucky. The forest topsoils and part of the subsoils were removed for use as fill in a large earthen dam in 1985. The original soils were classified as Shelocta gravely silt loams (Newton et al. 1973). Only the very strongly acid subsoils remain with a depth of approximately 30 cm over sandstone and shale. Three study areas were established on a broad second terrace above Red Lick Creek below a long east-facing slope with an open southern exposure. The site can be classified as xeric because of its exposure and shallow soil parent materials, although water from upper slope positions sometimes reaches the plots during and after high precipitation events.

In March 1986, the borrow area was chisel-plowed to a depth of 25 cm and 10-10-10 fertilizer was applied at a rate of 1210 kg ha^{-1}. Three study areas were subdivided into eight 3 m x 3 m plots in line with a 1 m buffer strip separating the plots. Forest topsoil to a depth of about 15 cm was collected from a nearby undisturbed Virginia pine-red maple (Pinus virginiana-Acer rubrum) stand using a farm tractor with a front-mounted end loader and deposited adjacent to two of the study areas. Topsoil was similarly collected for use in a third area from an adjacent American beech-white oak (Fagus grandifolia-Quercus alba) stand.

The study was laid out as four independent experiments to study 1) effectiveness of topsoil for introducing native species, 2) effect of the reclamation species on native species, 3) effects of different methods of topsoil use, and 4) effects of mulch. Eight treatment plots were randomly assigned and replicated once in each of three blocks in the barrow area. Treatments were as follows:

- T1. 1 cm topsoil plus mulch.
- T2. 1 cm topsoil lightly tilled into soil with a rototiller plus mulch.
- T3. 2 cm topsoil plus mulch.
- T4. 1 cm topsoil minus mulch.
- T5. 1 cm topsoil equivalent applied in two strips 4 cm deep across the plot plus mulch.
- T6. 1 cm topsoil plus a reclamation species mix plus mulch.
- T7. No topsoil, reclamation species mix only plus mulch.
- T8. No topsoil, no reclamation mix, mulch only.

The required amounts of topsoils were spread April 4 on each plot by filling buckets with the appropriate volumes of topsoil, transferring the soil to the plot, and raking it evenly or stripping it over the surface. The reclamation species mix was seeded on April 9. It consisted of Japanese lespedeza (Lespedeza striata) seeded at 9 kg ha^{-1}, Korean lespedeza (Lespedeza stipulacea) at 8 kg ha^{-1}, white clover (Trifolium repens) at 5 kg ha^{-1}, perennial ryegrass (Lolium perenne) mixed with Italian ryegrass (L. multiflorum) at 9 kg ha^{-1}, orchard grass (Dactylis glomerata) at 11 kg ha^{-1}, and timothy (Phleum pratense) at 4 kg ha^{-1}. A common wheat (Triticum aestivum) hay mulch was spread at the rate of 2 t ha^{-1} on the day of seeding. The surrounding borrow area was reclaimed at the same time in the same manner as the reclamation mix only plots but tall fescue (Festuca arundinacea) and red clover (Trifolium pratense) were added in some areas. Plots in which reclamation species were not desired were covered with plastic sheeting during the short time that seed was broadcast near the experimental areas.

Vegetation development was monitored and species presence in each plot was recorded at three to four week intervals. Cover after ten weeks was determined on June 20, 1986, using a pindrop method through a 20 point frame placed at random five times in each plot. Woody species were counted in each plot in late September, 1986. Vegetation development was recorded at various times using black and white or color photography. Nomenclature for all species follows Strausbaugh and Core (1978).

Data were analyzed on a Data General MV-7800 computer using the SYSTAT statistical program package of Wilkinson (1988). Data for numbers of native species, number of trees and number of shrubs were log transformed (n=log(n+1)) to reduce positive skewness, but data for other variables did not require this procedure. Comparisons of treatment means of vegetation variables were made using one-way analysis of variance (ANOVA) and Fisher's (protected) LSD (Steel and Torrie 1980). Independent variables tested by ANOVA were 1) use of topsoil,

2) use of reclamation mix, 3) use of mulch, and 4) method of topsoil use. Topsoil source was not considered as a variable. We decided a priori to make multiple comparisons of vegetation variables only among certain treatments as follows:

Absolute effectiveness of topsoil on vegetation variables:

T1 vs. T8,

Effects of reclamation mix on vegetation variables linked to topsoil:

T1 vs. T6 vs. T7

Effects of different methods of topsoil use on vegetation variables:

T1 vs. T2 vs. T3 vs. T5

Effects of mulch on vegetation variables:

T1 vs. T4

Significance level for all statistical tests was $\alpha = 0.05$. Analysis of variance tables are presented in Appendix 1.

Rainfall amounts were measured and recorded at the Northeastern Forest Experiment Station, Forestry Sciences Laboratory, about 3 km from the study site.

Results and Discussion

This study revealed that small quantities of forest topsoils and their seed banks can be used successfully to introduce native species to borrow areas. However, the most obvious differences in plots were linked to use of the reclamation species mix and the hay mulch at ten weeks after establishment and afterwards. Where the reclamation mix was employed, an aggressive cover of planted grasses dominated the plots with lesser amounts of annual legumes. Where mulch was employed without the reclamation mix, the plots were dominated by wheat and timothy. Scattered native species were established in all topsoiled plots that did not have the seeded reclamation mix.

Forest topsoils significantly increased the number of native or naturalized plant species in the plots during the first and second growing seasons (one-way ANOVA of Treatment 1 and Treatment 8 data, $\alpha = 0.05$) (Table 1). Most of these species were few in number and occurred in only a few plots. In all topsoiled plots, 57 native species were found during the first growing season. The mean number of native or naturalized species per topsoiled plot without the reclamation mix was 11 with a range of 6 to 20. During the second year, a total of 87 species were documented with a mean of 19 and a range of 7 to 27 native or naturalized species per topsoiled plot. Some species found the first year were not present during the second growing season. Nonplanted native or naturalized species totaled 90 taxa from 34 families present in either or both years (Table 2). This number of established species is comparable to the 199 species found in a greenhouse study of eight Appalachian forest seed banks (Dobberpuhl 1981), 134 species from three Appalachian forest topsoils spread over mine spoils (Farmer et al. 1982), and 84 species in one forest topsoil seed bank spread on mine spoil in irrigated microplots (Wade 1986, 1989).

Native species composition of topsoiled plots was characteristic of the early seral stages of secondary forest succession, but species composition and frequency were not comparable to the standing forests (intermediate or late seral stages) of the topsoil donor sites.

Topsoiling resulted in a significantly greater increase in the mean numberof species in the plots (ANOVA) of Treatment 1 and Treatment 8 data, $\alpha = 0.05$ (Table 1). This may have been because additional dormant seed in the applied soils did not germinate until the second year. Other possibilities are that invaders were more successfully established after modification of environmental factors, or the presence of symbiotic soil microflora, especially mycorrhizal fungi. Farmer et al. (1982) observed that many native species from forest topsoil seed banks on newly exposed mine spoils had endomycorrhizal associates. Korean and Japanese lespedezas in the reclamation mix were observed to be growing more luxuriantly on and near the topsoil strips used in Treatment 5 than in the rest of the plots. This may have been because of native strains of *Rhizobium* spp. in the topsoils. Although the amounts of added forest soils were small, fertility factors might also have been influential in facilitating greater establishment of native species on topsoils.

Topsoil application significantly increased the number of shrubs but not the number of trees (ANOVA of Treatment 1 and Treatment 8 data, $\alpha = 0.05$) (Table 1). A total of seven tree species and seven shrub or woody vine species were present in all topsoiled plots (Table 2) with a mean total number of woody stems equivalent to 11,000 ha^{-1}. Of the trees, black locust (*Robinia pseudoacacia*) was the most numerous and fastest growing species and it was more frequent in the plots that received topsoil from the Virginia pine-red maple stand.

Native species from the seed bank did not provide a significant amount of ground cover in the plots after twn weeks in the first growing season (ANOVA of Treatment 1 and Treatment 8 data, $\alpha = 0.05$) (Table 1). Mean cover of native species in all topsoiled plots was 5 percent, but this was highly variable. The pin drop method of cover estimation is conservative since the fine pins as used in this study frequently drop through the canopies of some plants without actually touching leaves or stems. The amount of cover produced by seed bank species alone would not have met the standards for mined-land reclamation in the United States. Other forest topsoil seed bank studies in the eastern United States have produced acceptable cover during the first growing season under optimal growing conditions (Farmer et al. 1982; Wade 1986, 1989).

The reclamation mix species effectively eliminated wheat and timothy from the mulch and the native species in the topsoil (Table 3). ANOVA and Fisher's (protected) LSD of vegetation variable means from Treatment 1 (1 cm topsoil plus mulch), Treatment 6 (1 cm topsoil plus mulch and reclamation mix significantly ($\alpha = 0.05$) reduced the number of native species during the first two growing seasons, the number of trees, and the number of shrubs (Table 1). Cover of reclamation species was significantly higher than cover of wheat and timothy from the mulch. There were no significant differences in the amount of native species cover because of the low but highly variable numbers of native species in topsoiled plots. The increase in native species between the first and second years was significantly less in plots containing the reclamation mix. This suggests that the reclamation mix community could retard plant succession.

Table 1. Effects of various factors on vegetation variables in Cowbell Hollow plots. Within each factor group, means followed by the same letter are not significantly different, ANOVA followed by LSD where appropriate, α = 0.05.

Factor	Treat.	Year 1 Native Species	Year 2 Native Species	Incr. Native Species	Number Trees	Number Shrubs	% Cover	% Cover Native
Topsoil	T1	8.7a	22.0a	13.3a	11.3a	3.7a	71.3a	4.0a
	T8	0.7b	5.0b	4.3b	0.7a	0.0b	76.0a	0.3a
Rec Mix	T1	8.7a	22.0a	13.3a	11.3a	3.7a	71.3b	4.0a
	T6	0.3b	3.3b	3.0b	0.0b	0.3b	100.0a	0.0a
	T7	0.3b	3.0b	2.7b	0.3b	0.0b	96.3a	0.0a
Topsoil Method	T1	8.7a	22.0a	13.3a	11.3a	3.7a	71.3a	4.0a
	T2	8.3a	14.7a	6.3a	2.0a	0.3a	72.3a	7.0a
	T3	11.0a	20.0a	9.0a	15.0a	3.7a	81.0a	5.3a
	T5	8.7a	18.0a	9.3a	5.3a	2.7a	66.0a	5.0a
Mulch	T1	8.7a	22.0a	13.3a	11.3a	3.7a	71.3a	4.0a
	T4	16.0a	19.0a	3.0b	16.0a	4.3a	9.0b	4.7a

Table 2. Frequency of species occurrence in plots of each treatment in 1986 and 1987.

	T1		T2		T3		T4		T5		T6		T7		T8	
	TS		TS til		TS 2x		TS-mul		TSstri		TS+RM		RMonly		No TSRM	
SPECIES	86	87	86	87	86	87	86	87	86	87	86	87	86	87	86	87
Acalypha gracilens Gray	.3		.3		.3		.6		.3							
Acalypha rhomboidea Raf.		.7				.7										
Acer rubrum L.		.7		.7				.3	.7			.3		.3		
Agrostis perennans (Walt.) Tuckerm.								.3								
Aster pilosus Willd.						.7				.3						
+*Bromus japonicus* Thunb.														.3		.3
+*Cardamine hirsuta* L.		.3														
Carex digitalis Willd.		.3		.3						.3						
+*Cerastium viscosum* L.		.3														
Chenopodium album L.													.3			
+*Chrysanthemum leucanthemum* L.			.3													
+*Cirsium arvense* L.				.7		.7			.3							
+*Cirsium vulgare* (Savi) Ten.			.3	.3												
Cyperus esculentus L.									.3							
+**Dactylis glomerata* L.				.3		.3						.3		.3		.3
Danthonia spicata L.								.3								
Desmodium paniculatum (L.) DC.	.3															
+*Digitaria ischaemum* (Schreb.) Muhl.			.3		.3			.3								
+*Digitaria sanguinalis* L.		.3	.3													
Diodia teres Walt.							.3									
Eragrostis spectabilis (Pursh) Steud.		.3														

Table 2. Frequency of species occurrence in plots of each treatment in 1986 and 1987. (continued)

	T1		T2		T3		T4		T5		T6		T7		T8	
	TS		TS til		TS 2x		TS-mul		TSstri		TS+RM		RMonly		No TSRM	
SPECIES	86	87	86	87	86	87	86	87	86	87	86	87	86	87	86	87
Erechtites hieracifolia (L.) Raf.	.7		.3		.7		.7									.3
Erigeron annuus (L.) Pers.		1.		1.		.3	.3	.7		1.		.7		.3	.3	.7
Erigeron canadensis L.		1.		1.		1.	.3	.7		1.		.3		.3		.7
Erigeron philadelphicus L.		.3					.3			.3						
Erigeron strigosus Muhl.		.7														
+Erucastrum gallicum (Willd.) O. E. Shulz			.7				.7	.3	.3	.3						
Eupatorium serotinum Michx.	.7	.7		.3	.7	.7	.7	1.	.7	.7						
+*Festuca arundinacea Schreb.	.7	1.	1.	.7	1.	1.	.7	1.	1.	1.	1.	1.	1.	1.	.7	1.
Fragaria virginiana Duchesne.		.3			.3	.3										
Geranium caroliniana L.				.3		.3		.3								.3
Gnaphalium purpureum L.		.7		.7		.3		.3								
Hedeoma pulegioides (L.) Pers.			.3	.3					.3							
Helianthus microcephalus T. & G.	.3				.3	.3										
Hieracium venosum L.							.3									
Houstonia purpurea L.		.3						.3								
Hypericum gentianoides (L.) BSP.		.3			.3					.3		.3				
Hypericum punctatum Lam.		.3						.3								
+Ipomoea hederacea (L.) Jacq.										.3						
Iva ciliata Willd.										.3						
Juncus tenuis Willd.	.3	1.		.3		1.	.7	.7	.3							
Lactuca canadensis L.	.3			.3		.7										
+Lactuca serriola L.		.7	.3	.7		.3		.3		.7						
Lespedeza intermedia (S. Wats.) Britton							.3									
Lespedeza repens (L.) Bart.						.3										
+*Lespedeza stipulacea Maxim.	1.	1.	1.	.7	1.	1.	1.	1.	1.	1.	1.	1.	1.	1.	1.	1.
+*Lespedeza striata (Thunb.) H. & A.		.3		.7				.7	.3							
Lindernia dubia (L.) Pennell									.3							
Liriodendron tulipifera L.	.3	.3				.3	.3	.3		.3						
Lobelia inflata L.						.7	.7									
+*Lolium multiflorum Lam.	.7	1.	.7	1.	1.	1.	.3	1.	1.	1.	1.	1.	1.	1.	1.	1.
+*Lolium perenne L.	1.	.7	.3	.7	1.	1.	.3	1.	1.	1.	1.	1.	1.	1.	1.	1.
Lysimachia quadrifolia L.						.3		.3								

Table 2. Frequency of species occurrence in plots of each treatment in 1986 and 1987. (continued)

	T1		T2		T3		T4		T5		T6		T7		T8	
	TS		TS til		TS 2x		TS-mul		TSstri		TS+RM		RMonly		No TSRM	
SPECIES	86	87	86	87	86	87	86	87	86	87	86	87	86	87	86	87
+Medicago lupulina L.	.3	.3	.3	.3		.3			.7	1.		.7				
+Microstegium vimineum (Trin.) A. Camus							.3									
Panicum clandestinum L.								.3								
Panicum commutatum Schultes					.7			.3								
Panicum dichotomum L.					.3		.3									
Panicum lanuginosum Ell.				.3	.3	.7	.3	.7								
Panicum polyanthes Schultes								.3								
Paronychia fastigiata (Raf.) Fernald	.3		.3		.3											
+*Phleum pratense L.	.7	.7	.7	.7	.7	.3	.3	.7	.3	.3	.3		.3		.7	.3
Physalis heterophylla Nees.								.3								
Phytolacca americana L.	.7	.7	.7		1.	.7	1.	.7	.7	.7					.3	
Pinus virginiana Mill.		.3				.3					.3					.3
Plantago rugelii Dcne.				.3			.3	.7								.3
Platanus occidentalis L.							.3	.3								
+Poa compressa L.								.3								
Polygonatum biflorum (Walt.) Elliott		.3				.3				.3						
Polygonum scandens L.	1.	.7	.3	.3	.7	.3			.7	.7				.7	.3	.3
Populus deltoides Marsh.								.3								
Potentilla norvegica L. Ann.										.3						
+Potentilla recta L. Per.				.3						.3						
Potentilla simplex Michx.										.3						
Rhus copallina L.	.3	1.			.7	.3	1.	1.	.7	.7						
Rhus glabra L.	.7	.7	.3	.3	.3	.3	.7	.3	.3	.3						
Robinia pseudoacacia L.	.7	.7	.7	.7	1.	1.	1.	1.	1.	1.	.3					
Rubus allegheniensis T. C. Porter		.3	.3	.3		.3	.3	.7	.3	.3						
+Rumex crispus L.			.3	.3					.3	.3	.3	.3				
Sassafras albidum (Nutt.) Nees				.3		.3	.3	.3	.3	.7	.3	.3				
Scutellaria leonardii Epling			.3	.3			.3	.3								
+Sida spinosa L.			.7	.3			.3									
Smilax glauca Walt.										.3		.3				
Smilax bona-nox L.												.3				
Solanum carolinense L.		.3		.7	.3	.3										
Solanum nigrum L.	.3	.3			.3				.3	.3						
Solidago canadensis L.	.3	.3						.3		.3						
+Sonchus asper (L.) Hill.						.3		.3								
+Sonchus oleraceus L.			.7		.3	.3	.3	.3	.3	.3						
+Taraxacum officinale Weber.		.3	.3	.7		.3	.3	.7		1.				.3		
+Trifolium campestre Schreb.				.3												

Table 2. Frequency of species occurrence in plots of each treatment in 1986 and 1987. (continued)

	T1		T2		T3		T4		T5		T6		T7		T8	
	TS		TS til		TS 2x		TS-mul		TSstri		TS+RM		RMonly		No TSRM	
SPECIES	86	87	86	87	86	87	86	87	86	87	86	87	86	87	86	87
+**Trifolium pratense* L.	.3		.3		.3				.3							
+**Trifolium repens* L.		.3			.3	.7		.3	.3	.3						
+**Triticum aestivum* L.	1.	1.	1.	1.	1.	1.		.7	1.	1.	1.	1.	.7	.7	1.	.7
+*Verbascum blattaria* L.										.3						
+*Verbascum thapsus* L.			.3	.3		.3	.3									
+*Veronica arvensis* L.	.3															
Viola affinis Le Conte.					.3	.7										
Vitis aestivalis Michx.	.3						.3	.3								

* Planted in reclamation mix plots and in the surrounding area.

\+ Naturalized species.

Table 3. Mean number of native species present per plot in the first and second years, total number of trees, total number of shrubs, percent cover (at 10 weeks), and percent cover by native or naturalized species, planted grasses, planted legumes, and wheat. Standard deviations are in parentheses.

	TREATMENT							
	TS 1 cm	TS 1 cm Tilled	TS 2 cm	TS no mulch	TS strip	TS + RecMix	RecMix only	no TS or RecMix
# native spp. year 1	8.7 (3.8)	8.3 (2.1)	11.0 (4.4)	16.0 (6.1)	8.7 (4.6)	0.3 (0.6)	0.3 (0.6)	0.7 (0.6)
# native spp. year 2	22.0 (2.0)	14.7 (7.1)	20.0 (9.6)	19.0 (8.5)	18.0 (3.5)	3.3 (2.5)	3.0 (2.7)	5.0 (1.7)
2nd year ssp. increase	13.3 (4.0)	6.3 (5.0)	9.0 (7.2)	3.0 (3.5)	9.3 (4.2)	3.0 (3.0)	2.7 (2.9)	4.3 (2.1)
# trees	11.3 (12.9)	2.0 (1.0)	15.0 (19.1)	16.0 (19.2)	5.3 (3.5)	0.0 (0.0)	0.3 (0.6)	0.7 (0.6)
# shrubs	3.7 (3.1)	0.3 (0.6)	3.7 (3.1)	4.3 (3.5)	2.7 (2.5)	0.3 (0.6)	0.0 (0.0)	0.0 (0.0)
% cover	71.3 (11.7)	72.3 (25.7)	81.0 (12.3)	9.0 (9.8)	66.0 (6.6)	100.0 (0.0)	96.3 (4.0)	76.0 (4.6)
% cover native species	4.0 (5.3)	7.0 (3.0)	5.3 (4.0)	4.7 (2.3)	5.0 (8.7)	0.0 (0.0)	0.0 (0.0)	0.3 (0.6)
% cover planted grasses	4.0 (6.9)	0.7 (1.2)	0.0 (0.0)	0.0 (0.0)	0.7 (1.2)	100.0 (0.0)	96.3 (4.0)	0.0 (0.0)
% cover planted legumes	0.3 (0.6)	0.3 (0.6)	0.7 (1.2)	0.0 (0.0)	0.7 (1.2)	7.3 (5.8)	6.0 (2.6)	0.0 (0.0)
% cover wheat	60.7 (12.2)	68.3 (32.3)	75.7 (19.0)	5.0 (7.8)	52.0 (9.5)	7.3 (5.1)	4.3 (5.9)	75.7 (4.5)

Comparisons of different methods of topsoil use: 1 cm, 1 cm tilled into the substrate, 2 cm, and a 1 cm equivalent piled in deeper strips, showed no significant differences among methods of topsoil use for any vegetation variables (ANOVA, $\alpha = 0.05$) (Table 1).

Use of mulch resulted in a significantly greater increase in native species and cover (ANOVA of Treatment 1 and Treatment 4 data, $\alpha = 0.05$) (Table 1). The cover difference was largely because of wheat and timothy from seed input with the mulch. Greater increase in native species in mulched plots probably occurred because mulch conserved soil moisture, lowered surface temperatures, provided a trapping effect on wind-blown seeds, and reduced erosion. Mulch did not significantly affect the number of native species in the first two years, cover in the first year, or the total number of trees or shrubs. These results suggest that with seed-free straw mulches a light seeding of wheat or timothy may be desirable to provide additional cover in the first growing season of native species introduced via seed banks.

Native species were established during a severe drought on an exposed site with shallow soils and without protective cover except the hay mulch. Total precipitation was 0.7 and 1.9 standard deviations below normal in 1986 and 1987, respectively. Water deficits during most months of the growing seasons were even greater (Table 4). While the wheat and timothy from the mulch provided some shading and amelioration of surface temperatures, they were also competitors for soil water.

In a study using a forest topsoil seed bank spread over two surface-mine spoils, the number and biomass of established native species was significantly reduced when a reclamation mix of Italian ryegrass (Lolium multiflorum), perennial ryegrass (L. perenne), tall fescue (Festuca arundinacea), weeping lovegrass (Eragrostis curvula), and sericea lespedeza (Lespedeza cuneata) was seeded into the forest topsoil, but the native species were not eliminated (Wade 1986, 1989). Ground cover developed fastest in the reclamation mix and slowest in the seed bank community, but there were no significant differences in ground cover 16 weeks after establishment. Doerr and Redente (1983) also observed that a mixture of seeded grasses repressed native forb biomass production in Colorado. The lower cover and populations of native species in the Cowbell Hollow borrow area may be attributed to the harsher environmental conditions, especially drought, low soil moisture, and possibly a reduced number of propagules in the smaller amount of forest topsoil used.

Conclusions

Forest topsoil seed banks can be used to introduce a variety of native species under harsh environmental conditions to drastically disturbed lands such as borrow areas. Many of the species contained in forest topsoil seed banks are not available from seed suppliers. For the reclamationist interested in native species establishment, topsoil seed banks provide a low cost, easily available propagule source for the revegetation of disturbed sites for enhancement of wildlife or forest habitats.

The coemployment of common, aggressive herbaceous ground cover species can significantly reduce success of native species establishment from forest topsoils. The reclamation species mix effectively eliminated all species from the forest topsoil seed banks during the first year; 82 were present during the second year. Different methods of forest topsoil use did not significantly affect results. Effects of hay mulch were not significant except that presence of mulch significantly enhanced native species establishment during the second year.

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Table 4. Growing season and yearly precipitation (mm) at Berea, Kentucky, for 1986, 1987, and the 1951-1980 mean and standard deviation (Conner et al. 1981).

Year	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Year Total
1986	110	71	29	67	45	102	86	156	1043
1987	90	73	49	58	73	82	32	11	813
mean	85	122	115	106	109	122	102	95	1193
s.d.	56	56	61	48	48	55	61	60	201

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Appendix 1. Analysis of variance tables for effects of topsoil use, reclamation mix, method of topsoil use, and mulch.

Source	SS	DF	MS	F	Sig.	Data
$\log_{10}$ number of species, 1st year						
topsoil	4.643	1	4.643	31.437	**	T1 T8
error	0.591	4	0.148			
rec mix	7.924	2	3.962	26.090	**	T1 T6 T7
error	0.911	6	0.152			
method	0.122	3	0.041	0.324	ns	T1 T2 T3 T5
error	1.004	8	0.125			
mulch	0.470	1	0.470	3.054	ns	T1 T4
error	0.615	4	0.154			
$\log_{10}$ number of species, 2nd year						
topsoil	2.830	1	2.830	50.541	**	T1 T8
error	0.224	4	0.056			
rec mix	6.779	2	3.389	12.552	**	T1 T6 T7
error	1.620	6	0.270			
method	0.330	3	0.110	0.687	ns	T1 T2 T3 T5
error	1.281	8	0.160			
mulch	0.065	1	0.065	0.549	ns	T1 T4
error	0.473	4	0.118			

Appendix 1. Analysis of variance tables for effects of topsoil use, reclamation mix, method of topsoil use, and mulch. (continued)

Source	SS	DF	MS	F	Sig.	Data
species increase						
topsoil	121.500	1	121.500	11.758	*	T1 T8
error	41.333	4	10.333			
rec mix	220.667	2	110.333	9.832	*	T1 T6 T7
error	67.333	6	11.222			
method	75.000	3	25.000	0.901	ns	T1 T2 T3 T5
error	222.000	8	27.750			
mulch	160.167	1	160.167	11.306	*	T1 T4
error	56.667	4	14.167			
log_{10} number of trees						
topsoil	4.090	1	4.090	5.893	ns	T1 T8
error	2.776	4	0.694			
rec mix	8.064	2	4.032	8.713	*	T1 T6 T7
error	2.776	6	0.463			
method	2.622	3	0.874	1.108	ns	T1 T2 T3 T5
error	6.308	8	0.789			
mulch	1.014	1	0.104	0.080	ns	T1 T4
error	5.181	4	1.295			
log_{10} number of shrubs						
topsoil	2.883	1	2.883	12.000	*	T1 T8
error	0.961	4	0.240			
recmix	3.3310	2	1.655	7.750	*	T1 T6 T7
error	1.281	6	0.214			
method	2.677	3	0.892	1.781	ns	T1 T2 T3 T5
error	4.008	8	0.501			
mulch	0.019	1	0.019	0.037	ns	T1 T4
error	2.110	4	0.528			
cover (percent)						
topsoil	32.667	1	32.667	0.413	ns	T1 T8
error	316.667	4	79.167			
rec mix	1460.007	2	730.003	14.252	**	T1 T6 T7
error	307.33	6	51.222			
method	347.333	3	115.778	0.468	ns	T1 T2 T3 T5
error	1979.333	8	247.417			
mulch	5828.167	1	5828.167	49.743	**	T1 T4
error	468.667	4	117.167			
native species cover (percent)						
topsoil	20.167	1	20.167	1.424	ns	T1 T8
error	56.667	4	14.167			
rec mix	31.947	2	15.973	1.711	ns	T1 T6 T7
error	56.00	6	9.333			
method	14.00	3	4.667	0.145	ns	T1 T2 T3 T5
error	256.667	8	32.083			
mulch	0.667	1	0.667	0.040	ns	T1 T4
error	66.667	4	16.667			

Recolonization of Vesicular-Arbuscular Mycorrhizae on a Reclaimed Strip Mine in North Dakota. John Reed Cockrell, Graduate Student, Dept. of Animal and Range Sciences, North Dakota State University, Fargo, ND 58105.

Vesicular-arbuscular mycorrhizae (VAM) are an integral component of North Dakota grassland ecosystems. Stockpiling topsoil in the process of mining lignite coal can drastically reduce the number of viable VAM propagules; reestablishment of VAM is therefore an important measure of reclamation success. Numerous soil parameters as well as topography and age of the reclaimed soil may all influence VAM recolonization. This study investigates the relationship of VAM infection to numerous soil properties, basal cover and time since reclamation. Thirteen transects of ten points each have been laid out on the Glenharold Mine near Stanton, North Dakota. Four transects have been placed on undisturbed sites, four on a site reclaimed in 1979 and five on a site reclaimed in 1984. Topographic data have been collected at each transect point, along with percent basal cover of plant species and numerous soil properties: pH; electrical conductivity; saturation percentage; calcium magnesium, sodium and sodic adsorption ratio (SAR); and sand, silt and clay percentages. Root samples of Agropyron smithii (a cool season grass) and Bouteloua gracilis and B. curtipendula (warm season grasses) were excavated at each transect point, and stained, and analyzed under a microscope to determine percent mycorrhizal infection. Preliminary data indicate that VAM infection is significantly higher on the reclaimed sites than it is on undisturbed sites, for both cool season and warm season species. Undisturbed sites exhibit VAM infection levels of 7.60 ± 0.52% for Agropyron and 6.58 ± 0.50% for Bouteloua, while the site reclaimed in 1979 exhibits 24.43 ± 0.88% infection for Agropyron and 17.43 ± 1.20% for Bouteloua. The site reclaimed in 1984 exhibits 15.57 ± 0.66% VAM infection for Agropyron. There appears to be little, if any correlation between infection level and basal cover or soil properties. No trends in infection level due to topography have been noted.

exhibits a wide range of chemical characteristics. Such materials often represent an environmental hazard because of toxic substances that can develop in and leach from them. Recently enacted laws (Surface Mining Control Act of 1977) require that all permanent refuse disposal sites be vegetated with a permanent self-sustaining plant cover.

The inherent low fertility and recent deposition of coal refuse presents a set of growth-limiting factors similar to those encountered by pioneer plant species. Advancing on this premise, several studies (Schramm 1966; Daft and Hacskaylo 1976; Khan 1978) have been conducted to determine possible adaptations by successful colonizing species to overcome the stresses presented.

Schramm (1966) concluded that plants successfully colonizing bare, nutrient-deficient coal wastes are generally nitrogen-fixing, mycorrhizal, or both. A majority of plants growing on coal refuse piles in Scotland (Daft and Hacskylo 1974) and Australia (Khan 1978) was found to be infected by mycorrhiza-forming fungi.

The benefit to the "host plant" in a mycorrhizal association is widely believed to be due to improved phosphorus nutrition of the host (Daft and Hacskylo 1976; Lambert and Cole 1980). The possible alteration of other physiological processes that would enhance colonization of stressful sites has also been suggested (Schramm, 1966; Khan 1978).

A major purpose of the studies presented in this work was to examine the possible links between successful plant colonization of coal-refuse piles and the presence of vesicular-arbuscular mycorrhizae (VAM). In many situations, the presence of VAM does not confer an advantage to the host plant (Hetrick et al. 1984); i.e., VAM infection cannot always be viewed as beneficial. While the VAM-host-plant interaction may be highly integrated both structurally and physiologically, better growth of the host is not automatically a consequence of the relationship. Host-plant responses vary widely when conditions of nutrition, soil, and other environmental factors are changed. Isolates (ecotypes) of the same VAM species collected at locations with differing rainfall patterns have been shown to have substantially different effects on host-plant water relations (Stahl and Smith 1986). Especially at the species level, the presumption that differing host-plant responses would occur seems valid.

The ability of a VAM association to enhance host plant growth has been called symbiotic efficiency (Smith and Gianinazzi-Pearson 1988). Several growth parameters have been used to quantify the symbiotic efficiency of a VAM isolate with a particular host species. In situations of environmental stress good indicators of symbiotic efficiency are relative growt rate and root:shoot ratio (Smith an Gianinazzi-Pearson 1988). Unde nutritional stress, possible determinant of symbiotic efficiency of a VAM isolate o species are host nutrient uptake an deployment as measured by tissue analysi (Smith and Gianinazzi-Pearson 1988).

A central (implicit) hypothesis in thes studies is that VAM species isolated fro environments most closely matching th experimental conditions of a study, wil exhibit a higher degree of symbioti efficiency. Commonly, VAM isolates ar selected for their ability to promot growth under non-stressful conditions. B selecting ecotypes that have evolved on o near refuse piles the possibility o finding a VAM-producing fungus that wil enhance its hosts tolerance of thes stresses is much improved.

Experimental Methods

Seedling Establishment

White clover (Trifolium repens) seedling were established in plastic cone-tainer that had been filled with various mixture of soil and coal refuse. The soil, Groseclose silt loam, was mixed with coa refuse to achieve 0, 25, 50, 75 or 100 coal refuse content. Nutrient status ar pH for the five soil:refuse combinatior is shown in Tables 1 and 2. Prior t mixing, soil was fumigated with methy bromide and thoroughly aerated. Coa refuse samples taken from several seams i the Pocahontas formation of West Virgini were used after being autoclaved at 180 for 30 minutes, allowed to cool for 18 hou and re-autoclaved. In preparation for thi experiment, the coal refuse was treate with lime at 2 Mg/ha 18 months prior t experimentation.

At planting, soil preinoculated wit either Glomus etunicatium (Ge) or Glomu sp. (Gs) or filtrate from sievings of th two spore cultures (none) was added to eac cone-tainer. After seedling emergence, bu prior to the appearance of the firs trifoliolate leaf, plants were thinned t one per pot.

Table 1. Chemical characteristics of soi coal refuse mixtures.

Growth Media	pH	E.C.
% Refuse		dS/cm
0	5.6	0.22
25	6.8	0.23
50	7.3	0.34
75	7.4	0.42
100	7.4	0.69

Table 2. Plant available nutrient content of soil:coal refuse mixtures.

Growth Media	NH4OAC Extractable P	K	Ca
% Refuse	------- mg/kg --------		
0	3.8	47.4	216.0
25	4.8	46.8	412.0
50	7.6	57.4	648.0
75	7.4	64.4	777.6
100	9.4	68.2	871.2

Plants were grown in a growth chamber under a 14 h daylength, temperatures of 25° C day /18oC night and relative humidities of 70 to 80 %. All plants were irrigated with 5 ml of 50% Hoaglands nutrient solution minus P - containing salts (Hoagland and Arnon, 1950) daily. At biweekly intervals, all mycorrhizal treatments were fertilized with 10 ml of 50% Hoaglands nutrient solution with P salts added, non-mycorhizal treatments received 10 ml weekly.

Water Relations Studies

When the plants were 55 days old, all pots were brought to field capacity and wrapped to prevent evaporative water loss, and water was withheld for five days. Relative water content (RWC)(Matin et al. 1989) was determined for an excised leaf at day five. Determination of RWC was performed using the formula:

$$RWC = [(FW - DW)/(TW - DW)]* 100$$

where FW = fresh weight
DW = dry weight
TW = turgid weight (after rehydration in distilled water).

Post-Harvest Measurements

Plants were removed from all pots 60 days after planting, and the soil:refuse materials were washed from roots. Root length was estimated using the line intersect method (Tennant 1975). Roots and shoots were dried separately at 70 C for 18 hours and weighed. Rehydrated roots were stained (Kormanik et al. 1980) and the VAM colonization assessed by a modified line-intersect method (Biermann and Lindemann 1981). Concentrations of P in dried leaves was determined using inductive coupled plasma spectroscopy (Donahue 1986). Statistical analysis was conducted using a split-plot design. Data compilation was performed using GLM (General Linear Models) procedure in SAS (SAS Institute, Inc. 1985).

Results

When compared to the non-VAM control, total biomass production of white clover (Tables 3 and 4) was enhanced by the presence of VAM at all soil:refuse combinations. Top growth was particularly enhanced by VAM. The isolate Ge, a ubiquitous mycorrhizal species, produced higher shoot dry weights at 0 and 25% mixtures of coal refuse. Shoot biomass was greater with Gs when coal refuse contents were greater than 50%. Root biomass production was enhanced with VAM infection at higher coal refuse contents. In general, root biomass was greater in Gs infected plants where coal refuse was added to the growth medium.

Table 3. Root production as influenced by various soil refuse mixtures and two mycorrhiza (Glomus etunicatum (Ge) and Glomus sp. (Gs)).

Growth Media	VAM Symbiont Ge	Gs	None	LSD (0.05)
% Refuse	----- dry wt.(g) -----			
0	0.92	0.59	0.45	0.91
25	1.09	1.50	0.61	1.34
50	0.75	2.38	0.47	0.48
75	0.60	0.74	0.55	0.48
100	0.46	0.62	0.44	0.39
LSD (0.05)	0.96	0.96	0.36	

With increasing content of coal refuse in the growth medium, VAM-infected plants responded differently in the partitioning of dry matter (Table 5). A decrease in root production by Gs-infected plants at 75

Table 4. Shoot production as influenced by various soil refuse mixtures and two mycorrhiza (Glomus etunicatum (Ge) and Glomus sp. (Gs)).

Growth Media	VAM Symbiont Ge	Gs	None	LSD (0.05)
% Refuse	----- dry wt.(g) -----			
0	1.49	3.38	0.85	2.92
25	1.93	4.96	1.06	2.89
50	3.52	3.39	0.85	1.91
75	3.97	2.68	0.84	2.64
100	3.01	1.87	0.50	1.84
LSD (0.05)	2.52	3.19	0.64	

and 100% refuse shifted the shoot:root