INVESTIGATING THE EFFECTS OF ALTERNATIVE RECLAMATION PRACTICES ON

RECENTLY RECLAIMED GRASSLANDS

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ABSTRACT

Surface mining operations in the Northern Plains result in complex grassland ecosystems being dismantled and later systematically reclaimed. Such processes can create long-term challenges with regards to the ecological recovery of reclaimed grasslands, prompting the need to find alternative reclamation practices that improve plant community dynamics and soil properties. We assessed both plant community characteristics (i.e., species richness, diversity, abundance, and community composition) and soil properties (i.e., penetration resistance and volumetric soil moisture) of reclaimed grasslands with alternative reclamation practices. Early findings showed that certain alternative reclamation practices may aid in the initial recovery of reclaimed grasslands by supporting desirable native plant communities and improving soil conditions. However, newly reconstructed landscapes are dynamic and are susceptible to change over time. Overall, we suggest continued monitoring of these reclaimed grasslands, and perhaps the use of supplemental management to maintain and/or enhance the current conditions.

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DEDICATION

I would like to dedicate my thesis to my parents Bill and Sue and sisters Leah and Caitlin. I would not be who I am or where I am without them and their unwavering support. They continuously encourage me to pursue my dreams and never allow me to give up. I am eternally grateful to belong to such a strong and loving family. I love you all.

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CHAPTER 1: ALTERNATIVE RECLAMATION PRACTICES SUPPORT EARLY ESTABLISHMENT OF NATIVE GRASSES AND INHIBIT INVASIVE GRASSES Abstract

Surface mining infrastructure and subsequent reclamation practices destroy heterogenous topoedaphic features that would otherwise support a diverse plant community. Additionally, homogenously reclaimed grasslands struggle to establish and sustain a desirable heterogenous plant community due to stresses attributed to exotic species invasion. The aim of this study was to determine how contemporary reclamation practices could promote re-establishment of a diverse native grassland while inhibiting Kentucky bluegrass (Poa pratensis; KBG), an exotic grass, invasion. Grasslands on two different lignite mines were reclaimed with four different topsoil depths and two seeding methods. Vegetation species composition and abundance and above and belowground biomass was estimated. Both seeding method and topsoil depths significantly influenced plant community composition, though the influence of topsoil varied by location. Establishment of native grass species was highest in sites planted directly to native and with deeper topsoil, and native grass species were negatively correlated with KBG establishment. Aboveground biomass was significantly greater in deeper soils while neither seeding method nor topsoil depth influenced belowground biomass. Initial findings reveal these reclamation effects were successful in establishing a native diverse plant community that can inhibit KBG establishment. These reclaimed grasslands are still in early stages of ecological recovery and will continue to be dynamic. However, moving forward additional maintenance practices may be required to sustain this plant community.

Introduction

Ecosystems are fundamentally complex due to interactions between fauna, flora and abiotic factors such as climate, natural disturbances, and topoedaphic features (i.e. soil type/depth and topography; (Green & Sadedin 2005; Peipoch et al. 2015). Grassland ecosystems, are characterized by their plant community's response to heterogeneous topoedaphic features such as soil depth, texture, structure and topographic position (Fuhlendorf & Smeins 1998). Development of such features takes thousands of years but creates unique physical, chemical, and biological components that affect abiotic and biotic interactions. The result of these interactions is a heterogeneous landscape created by the variability of plant structure, distribution, and composition (Fuhlendorf & Engle 2001). Unfortunately, these landscapes are undergoing varying degrees of homogenization due to fossil fuel extraction activities and the

The process of coal extraction through surface mining, and subsequent reclamation activities, result in large, permanently altered landscapes (Pauletto et al. 2016; Stumpf et al. 2016). Initial alterations occur during removal of existing vegetation and soil. Prior to excavation, soil surveys categorize soils as simply "topsoil horizon" or "subsoil horizon", based on growing medium quality (Performance Standards-Suitable Plant Growth Material, 1987). Consequently, unique edaphic features are removed from the landscape during excavation and original soil horizons blended together (DePuit 1983). Further alterations occur once reclamation practices begin. Current approved practices require stable soils and distinct soil horizons (SMCRA 1977) and promote the re-spreading of soil horizons to uniform depths and reconstructing a landscape, "... to the gentlest topography consistent with adjacent unmined landscape elements" (Surface Mining and Reclamation Operations, 1979). Despite short-term benefits (i.e., release of worst-case bonding) these reclamation standards present unique challenges for establishing and sustaining a diverse plant community. The obstacles associated with trying to establish and sustain a diverse plant community on these dramatically altered land are only amplified by the influence and pressure of exotic species.

Introduced species have several advantages which include an early spring phenology (Alpert et al. 2000; Smith and Knapp 2019), abundant and easily dispersed seeds (Alpert et al. 2000), and aggressive growth rates that promote self-colonization. Additionally, recently disturbed (e.g., mined) landscapes lack well-established native vegetation (Bohrer et al. 2017a). These factors culminate to create a conducive environment for invasion of introduced species which if left unchecked, could result in a homogenized plant community over time (Alpert et al. 2000). For example, Kentucky bluegrass (KBG; Poa pratensis) is an introduced grass species that can homogenize both intact grasslands (DeKeyser et al. 2013; Toledo et al. 2014; Limb et al. 2018; Kral- O'Brien et al. 2019) and reclaimed grassland sites (Bohrer et al. 2017b). Kentucky bluegrass' ability to homogenize a landscape is due in large part to its development of a thick, senesced layer of thatch (Kral-O'Brien et al. 2019), increasing its competitive edge over natives while promoting self-colonization (Bohlen 2006). Recent studies in the Northern Great Plains (NGP) determined that KBG was the most dominant species on 40-years' worth of reclaimed grasslands, with highest presence on older sites (Bohrer et al. 2017a). This change in plant community composition is gradual enough so that plant diversity standards can be satisfied for performance bond purposes, but long-term the landscape becomes increasingly more susceptible to homogenization. Preventing establishment of KBG may not be a realistic task but determining what reclamation practices can be utilized to suppress its growth should be further investigated.

Finding and investigating alternative reclamation practices needs to occur to successfully reclaim heterogenous grasslands (Christensen 1997). One alternative practice is the application of variable topsoil depths to the landscape. Previous studies investigating the influence of

variable topsoil depths have determined that deeper soil promotes greater biomass production and canopy cover; while shallower soil supports greater species diversity (Redente et al. 1997; Bowen et al. 2005; Buchanan et al. 2005; Schladweiler et al. 2005). These results suggest that variability in topsoil depths may encourage some of the heterogeneous communities inherent in intact grasslands. What has not been directly addressed is how these variable topsoil depths could affect non-native species like KBG, and how variable topsoil could impact reclaimed grasslands' plant community long-term. The first objective of our study is to quantify the influence of variable soil depths (and weed control seeding methods) on plant composition, canopy cover, and biomass production. Second, we will quantify how these different treatments have influenced the establishment of KBG. We hypothesize that plant diversity will be higher in shallower soils, while biomass production, canopy cover, and KBG presence will be higher in deeper soils. Depending on the information gathered, our goal from is this research is to supply mining companies with new reclamation practices that both meet standards for performance bond release and improving the long-term heterogeneous communities of reclaimed grasslands.

Methods

Site Description

We conducted our research on two active lignite coal mines, BNI Ltd and Coteau Properties Company- Freedom Mine; here after referred to as BNI and COT, respectively. The BNI project site is located approximately 6 km SE of Center, North Dakota USA (lat. 47° 3'28.88"N long. 101°18'16.66"W) and the COT project site is located approximately 5 km NW of Beulah North Dakota USA (lat. 47°19'57.89"N long. 101°48'28.99"W). The mine locations fall within the semiarid, mixed-grass prairie of the NGP ecoregion. Air temperature ranges from -11°C in January to 22°C in August (NDAWN 2020), and the site averages 150 days frost-free days (USDA-NRCS 2006). Average precipitation during the growing season is 355 mm (NDAWN 2020). Pre-mined soil largely consist of silt loam, loam, and silty clay loam complexes and post-mine soils are reclassified as "mined-land complex", which consists of a thin layer of loam covering bed rock down to 152 cm (USDA-NRCS 2021a).

Experimental Design

Strip mining at both sites occurred at least 10 years prior to the establishment of our experimental units. Active mining at COT occurred between 2008 and 2009; the area then persisted as an overburden pile until 2018 at which time the overburden pile was dismantled and reclamation activities began. Mining activities at the BNI location took place in 2000 after which the area became an ash disposal pit until 2014. Reclamation for this site began in 2017. Establishment of our experimental units occurred during active reclamation, opposed to occurring on previously reclaimed land. Treatments at both locations consisted of spreading topsoil in four distinctive depths (Figure 1.1 & 1.2) and application of two different seeding methods. Reclamation practices vary slightly and specific topsoil depths vary between both locations. These seeding methods took place over the course of two growing seasons. In the first season, plots were either planted with a native seed mix or to a cover crop. During the second season, the same native seed mix replaced the remaining cover crop plots after an application of glyphosate to reduce unwanted vegetation establishing from the seed bank.

BNI

This study location has 24, 0.4 ha plots in an incomplete block design with three replicates (Figure 1.1). The topsoil depths are 8, 15, 23, and 30 cm. Soil respread ended and seed bed preparation and planting began in late spring/early summer of 2018 where half of the treatment plots were directly planted to the native seed mix (Table 1.1) and the other half seeded to an oat cover crop. In late spring/early summer of 2019, planting of the native seed mix took place on all remaining cover crop sites without an herbicide treatment. However, two of the 30

cm and three of the 23 cm sites on the western edge of the northern section experienced additional maintenance work in spring/early summer of 2019. This resulted in large swaths being reseeded, setting back some site's that were initially seeded directly to native sites in 2018. These alterations may have influenced some of the discrepancies found in the results, especially with regards to the 23 cm sites.



Figure 1.1. BNI Ltd treatment design layout. Solid colored blocks indicate treatments planted directly to the native seed mix and cross-hatched blocks indicate treatments planted first to cover crop (i.e., oats; Avena sativa) then to the native seed mix. Different colors represent each of the four separate topsoil depths.

Common Name	Scientific Name	Growth Form	kg pls/ha	% of Seed Mix	
Oats	Avena spp.	Graminoid	1.79	22.86	
Big Bluestem	Andropogon gerardi	Graminoid	0.56	7.15	
Blue Grama	Bouteloua gracilis	Graminoid	0.44	5.62	
Green Needlegrass	Nassella viridula	Graminoid	2.69	34.36	
Little Bluestem	Schizachyrium scoparium	Graminoid	0.67	8.56	
Sideoats Grama	Bouteloua curtipendula	Graminoid	0.45	5.75	
Switchgrass	Panicum virgatum	Graminoid	0.67	8.56	
Western Wheatgrass	Pascopyrum smithii	Graminoid	0.56	7.15	
Blanketflower	Gaillardia aristata	Forb*	0.11-0.36		
Hoary Vervain	Verbena stricta	Forb*	0.11-0.36		
Purple Prairie Clover	Dalea purpurea	Forb*	0.11-0.36		
Stiff Goldenrod	Solidago rigida	Forb*	0.11-0.36		
Wild Bergamot	Monarda fistulosa	Forb*	0.11-0.36		
Black-eye Susan	Rudbeckia hirta	Forb*	0.11-0.36		
Purple Cone Flower	Echinacea purpurea	Forb*	0.11-0.36		

Table 1.1. List of all species and amount of each species used in BNI seed mix. Includes both common and scientific names. An annual oat species (Avena spp.) was used as a cover crop.

* A minimum of 3 species are chosen among this list each year, depending on availability. Forbs account for 5% of the total seed mix.

COT

This study location has 24, 0.4 ha plots in a complete randomized block design with three replicates (Figure 1.2). The topsoil depths were 8, 15, 18, and 23 cm. Completion of soil work ended in late November 2018, resulting in the planting of all plots to an oat cover crop until the following spring. Half of the experimental plots remained as an oat cover crop and the other half sown to a native seed mix in April 2019 (Table 1.2). The remaining cover crop plots received an herbicide treatment of glyphosate (1.75 liter/ha) July, followed by the planting of the native seed mix.



Figure 1.2. Coteau Properties Company- Freedom Mine treatment design layout. Solid colored blocks indicate treatments planted directly to the native seed mix and cross-hatched blocks indicate treatments planted first to cover crop (i.e., oats; *Avena sativa*) then to the native seed mix. Different colors represent each of the four separate topsoil depths.

Table 1.2. List of all species and amount of each species used in COT seed mix. Incl	ludes both
common and scientific names. An annual oat species (Avena spp.) was used as a cov	er crop.

Common Name	Scientific Name	Growth Form	kg pls/ha	% of Seed Mix
Western Wheatgrass	Pascopyrum smithii	Graminoid	1.68	7.5
Slender Wheatgrass	Elymus trachycaulus	Graminoid	1.12	5
Green Needlegrass	Nassella viridula	Graminoid	2.24	10
Blue Grama	Bouteloua gracilis	Graminoid	2.24	10
Sideoats Grama	Bouteloua curtipendula	Graminoid	4.48	20
Switchgrass	Panicum virgatum	Graminoid	2.8	12.5
Little Bluestem	Schizachyrium scoparium	Graminoid	3.36	15
Prairie Sandreed	Calamovilfa longifolia	Graminoid	2.24	10
Sand Bluestem	Andropogon hallii	Graminoid	1.12	5
Big Bluestem	Andropogon gerardi	Graminoid	1.12	5

Data Collection

We established two, 60-meter transects in each of the 24 plots with a 10-meter minimum separation. Surveys of plant community composition and canopy cover occurred during peak production (mid-July) by placing a 0.5 x 0.5-meter frame every 10-meters along each transect. We identified every plant to a species level and estimated canopy cover using the following modified Daubenmire cover classes, 1 = trace -1%, 2 = 1-2%, 3 = 2-5%, 4 = 5-10%, 5 = 10-20%, 6 = 20-30%...13 = 90-95%, 14 = 95-98%, 15 = 98-99%, and 16 = 99-100% (Daubenmire, 1959). Midpoint values were used for analysis.

We classified each species based on their seasonality, metabolic pathway, and origin (native versus exotic) by referencing the USDA Plant Database (USDA-NRCS 2021b) and Minnesota Wildflowers (MN Wildflower 2021) websites. Following composition and canopy cover estimations the vegetation in each frame was clipped to 1 cm high to determine aboveground biomass. Biomass samples were dried at 150°C to a constant weight. Collection of belowground biomass (roots) occurred after peak production (early October). We chose three random locations within each of the 24 plots and took 3.8 cm soil cores to a depth of 30 cm with a FarmQA soil sampler implement. Root samples were washed, screened, and dried at 45 °C to a constant weight.

Statistical Analysis

We calculated the mean species cover and aboveground biomass weights by averaging all frames within each transect for each experimental plot. Additionally, we averaged the three belowground biomass samples per experimental plot and used this data to assess species richness and diversity and above/belowground biomass weights using analysis of variance (ANOVA) testing both main effects (i.e., topsoil depth and seeding method) and the interaction. Tukey's post-hoc procedures were used for means separation, followed significant ANOVA results. Data analysis utilized nonmetric dimensional scaling (NMDS) with Bray-Curtis distance measure and we ran permutational multivariate analysis of variance (PERMANOVA) to understand the relationship between plant community composition of each seeding method and topsoil depths (Oksanen 2019). Post-hoc tests were performed on each seeding method to assess differences in the plant community between topsoil depths. Plant functional trait data was then applied to our ordination as vectors to further describe the plant composition. Additionally, we fit linear regression models to assess correlations between KBG cover and native grass cover. For all our analyses we used R version 4.0.4 (R Development Core Team 2021) using car (Fox & Weisberg 2019), agricolea (Mendiburu 2020), vegan (Oksanen et al. 2019), and RVAideMemoire (Hervé 2021).

Results

Plant Community and Composition

We identified 51 and 37 plant species at BNI and COT, respectively. Six grasses and two forb species were established from the seed mixture at BNI. Seven grass species were established from the original mixture at COT. Average species richness at both locations was 14 with the number of species per treatment plot ranging between 15 and 12 at BNI and 15 to 13 at COT. Seeding method consistently showed correlations with stronger species richness and diversity, compared to the variable topsoil depths where results varied based on location. Both locations share similar trends regarding the influence of seeding method over species richness. However, both main effects were only significant at BNI, ($p_{seeding method} = 0.004$) and ($p_{topsoil depth} = 0.01$). The cover crop to native seeding method and 30, 15, and 8 cm of topsoil treatments had the most plant species (Figure 1.3). Similar to BNI, COT's cover crop to native seeding method and 8 cm of topsoil depth had the greatest number of species. Seeding method strongly influenced species diversity at both locations (both comparisons, $p \le 0.10$), but specific results differed by site. The cover crop to native seeding method had a greater plant species diversity at BNI (0.84) compared to treatment plots sown directly to native (Figure 1.4). Conversely, plots seeded directly to native had a higher plant species diversity at COT (0.83) (Figure 1.5). Topsoil depth had no significant effect on plant species diversity at either location (p>0.10).



Figure 1.3. Plant species richness as a function of topsoil depths and seeding methods at BNI. Bars denote one standard error. Means with same letter are not significantly different ($p \le 0.10$). Data were collected in July 2020 from BNI Coal, LTD near Center, ND USA.



Figure 1.4. Plant species diversity as a function of topsoil depths and seeding methods at BNI. Bars denote one standard error. Means with same letter are not significantly different (p<0.10). Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.



Figure 1.5. Plant species diversity as a function of topsoil depths and seeding methods at COT. Bars denote one standard error. Means with same letter are not significantly different (p<0.10). Data were collected in July 2020 from Coteau Properties Company- Freedom Mine near Beulah, ND USA.

Main effects influencing plant composition (species composition and abundance) differed between BNI and COT; functional groups aided in explaining primary drivers of species composition variability. A two-dimensional solution provided the best interpretation of the data and PERMANOVA analysis for BNI revealed seeding methods (p=0.001) and topsoil depths (p=0.001) explained plant species composition. However, interactions between seeding method and topsoil depth were not significant (p>0.10). Therefore, we focused further analysis at BNI on plots within soil depth separated by seeding method.

Initial analysis of seeding methods revealed that the greatest driver of community composition for BNI's seeding methods were C3 grass and forb species (Figure 1.6A). Regardless of seeding method, post-hoc testing showed that plant communities in 8 cm topsoil depths significantly differ from those in the 23 ($p\leq0.10$) and 30 cm ($p\leq0.10$) topsoil depths, and communities in the 15 cm depth significantly differed from those in the 30 cm ($p\leq0.10$) topsoil depths (Figure 1.6 B & C). Sites planted directly to native, short-lived perennials, native species, and all C3 species and C4 forbs primarily explained the variability on NMDS axis 1, while annual and perennial species and C4 grasses largely explained the variability on NMDS axis 2 ($p\leq0.10$) (Table 1.3). Plant compositional variability on NMDS axis 1 was largely explained by C3 species and C4 forbs, annual species, long and short-term perennials, and C4 grasses. Native and exotic species primarily explained plant composition variability on NMDS axis 2 for those sites planted to cover crop to native ($p\leq0.10$) (Table 1.3).

PERMANOVA analysis for COT showed seeding method alone (p=0.001) was significant in explaining plant composition (Figure 1.7). Sites seeded directly to native had only two drivers that explained variability of plant composition on either NMDS axis 1 or NMDS axis 2. Only C3 grasses was found to be significant on NMDS axis 1 (p \leq 0.10) (Table 1.4). Plant

composition variability on NMDS axis 1 was largely explained by C3 forbs, and C4 grasses and forbs, C3 grasses, annual and exotic species, and both native and exotic species primarily explained plant composition variability on NMDS axis 2 for those sites planted to cover crop to native ($p \le 0.10$).

Seeding method and topsoil depth both influenced KBG presence/cover at BNI ($p \le 0.10$), but not at COT (p > 0.10). Cover crop to native seeding method had more KBG canopy cover compared to those sites seeded directly to native (Figure 1.8). Kentucky bluegrass cover was higher in sites with 15 cm of topsoil than those with 23 and 30 cm of topsoil, but KBG cover was not different between 15 and 8 cm or 23 and 30 cm depths (Figure 1.8). Additionally, BNI's plant community composition shows a negative correlation with native grass species and KBG ($p \le 0.10$).



Figure 1.6. NMDS ordinations of plant community composition at BNI. Species composition of treatments planted directly to native and cover crop to native; k=2, stress = 0.17 (A) Species composition of treatments planted cover crop to native with ellipses representing topsoil depths; k=2, stress = 0.17 (B). Species composition of treatments planted directly to native with ellipses representing topsoil depths; k=2, stress = 0.17 (C). Vectors provide community composition associations given different metabolic/life-forms. Species code coloration is dictated by whether the species was in the seed bank (i.e., Species Not Seeded; black text) or part of the seed mixes (i.e., Species Seeded; grey text). Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.



Figure 1.6. NMDS ordinations of plant community composition at BNI (continued). Species composition of treatments planted directly to native and cover crop to native; k=2, stress = 0.17 (A) Species composition of treatments planted cover crop to native with ellipses representing topsoil depths; k=2, stress = 0.17 (B). Species composition of treatments planted directly to native with ellipses representing topsoil depths; k=2, stress = 0.17 (B). Species composition of treatments planted directly to native with ellipses representing topsoil depths; k=2, stress = 0.15 (C). Vectors provide community composition associations given different metabolic/life-forms. Species code coloration is dictated by whether the species was in the seed bank (i.e., Species Not Seeded; black text) or part of the seed mixes (i.e., Species Seeded; grey text). Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.

Table 1.3. Functional group correlation coefficients at BNI. Correlation coefficients for NMDS1 and NMDS2 broken down by seeding method revealing different topsoil depths. Bold values indicate whether a functional group had significant influence in explaining variability within each seeding method's plant community. Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.

Seeding Method	Functional Group Category	NMDS 1	NMDS 2	r ²	Pr(>r)
Directly to Native	Annual Species	0.4153	-0.9097	0.604	0.001
Directly to Native	Perennial Species	0.6879	0.7258	0.427	0.002
Directly to Native	Annual/Biennial Species	-0.7045	0.7097	0.183	0.105
Directly to Native	Short-lived Perennial Species	-0.9700	0.2430	0.589	0.002
Cover Crop to Native	Annual Species	0.9393	0.3430	0.377	0.011
Cover Crop to Native	Perennial Species	0.9993	0.0386	0.294	0.023
Cover Crop to Native	Annual/Biennial Species	0.0067	1.0000	0.144	0.194
Cover Crop to Native	Short-lived Perennial Species	-0.9615	-0.2748	0.650	0.001
Cover Crop to Native	Biennial Species	0.7134	0.7007	0.073	0.477
Directly to Native	C3 Grass Species	0.7826	0.6226	0.488	0.002
Directly to Native	C4 Grass Species	-0.4058	0.9140	0.278	0.039
Directly to Native	C3 Forb Species	-0.8887	-0.4585	0.386	0.011
Directly to Native	C4 Forb Species	0.8387	-0.5445	0.515	0.004
Cover Crop to Native	C3 Grass Species	0.9023	0.4311	0.380	0.009
Cover Crop to Native	C4 Grass Species	0.4527	-0.8917	0.609	0.001
Cover Crop to Native	C3 Forb Species	-0.9993	0.0367	0.551	0.001
Cover Crop to Native	C4 Forb Species	0.9506	0.3106	0.611	0.002
Directly to Native	Native Species	0.9367	0.3502	0.262	0.050
Directly to Native	Exotic Species	-0.8903	-0.4553	0.085	0.394
Cover Crop to Native	Native Species	0.68547	-0.72841	0.773	0.019
Cover Crop to Native	Exotic Species	-0.31528	0.949	0.312	0.019



Figure 1.7. NMDS ordinations of plant community composition at COT. Species composition of treatments planted directly to native and cover crop to native; k=2, stress = 0.21. Data were collected in July 2020 from Coteau Properties Company- Freedom Mine near Beulah, ND USA.

Table 1.4. Functional group correlation coefficients at COT. Correlation coefficients for NMDS1 and NMDS2 of different functional groups. Bold values indicate whether a functional group had significant influence in explaining variability in plant communities. Data were collected in July 2020 from Coteau Properties Company- Freedom Mine near Beulah, ND USA.

Seeding Method	Functional Group Category	NMDS 1	NMDS 2	r ²	Pr(>r)
Directly to Native	Annual Species	0.79940	-0.60080	0.231	0.072
Directly to Native	Perennial Species	0.07174	0.99742	0.126	0.239
Directly to Native	Short-lived perennial Species	-0.81958	-0.57297	0.184	0.173
Directly to Native	Annual/Biennial Species	0.97941	-0.20187	0.0028	0.969
Directly to Native	Other	-0.99654	0.08314	0.4381	0.005
Cover Crop to Native	Annual Species	-0.48558	0.87419	0.4327	0.005
Cover Crop to Native	Perennial Species	0.159	-0.98728	0.6529	0.001
Cover Crop to Native	Short-lived perennial Species	0.52943	0.84836	0.0364	0.667
Cover Crop to Native	Annual/Biennial Species	0.96487	0.26274	0.0672	0.495
Cover Crop to Native	Other	-0.03432	-0.99941	0.0126	0.886
Directly to Native	C3 Grass Species	0.60100	-0.79925	0.360	0.010
Directly to Native	C4 Grass Species	-0.19792	0.98022	0.215	0.067
Directly to Native	C3 Forb Species	-0.84687	0.53180	0.193	0.103
Directly to Native	C4 Forb Species	0.99656	0.08286	0.044	0.607
Cover Crop to Native	C3 Grass Species	-0.18720	-0.98236	0.442	0.001
Cover Crop to Native	C4 Grass Species	0.51784	-0.85548	0.408	0.003
Cover Crop to Native	C3 Forb Species	0.89279	-0.45047	0.491	0.002
Cover Crop to Native	C4 Forb Species	-0.19764	0.98027	0.436	0.002
Directly to Native	Native Species	0.81709	0.57651	0.0709	0.458
Directly to Native	Exotic Species	0.9953	-0.0968	0.0404	0.639
Cover Crop to Native	Native Species	0.017267	-0.99985	0.5302	0.001
Cover Crop to Native	Exotic Species	-0.19742	0.98031	0.4358	0.005



Figure 1.8. Percent canopy cover of Kentucky bluegrass (*Poa pratensis*) as a function of topsoil depth and seeding methods at BNI. Bars denote one standard error. Means with same letter are not significantly different (p<0.10). Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.

Biomass

Aboveground biomass had similar trends at both locations. Treatments seeded directly to native and/or applied with 30 cm of topsoil had the greatest amounts of aboveground biomass. However, main effects were only significant at BNI ($p \le 0.10$) (Figure 1.9). We found no trend in belowground biomass at either location for topsoil depth. Neither seeding method nor topsoil depth had an effect at COT. However, at BNI seeding directly to native supplied significantly higher belowground biomass than the cover crop to native seeding method ($p \le 0.10$). Additionally, biomass on shallower topsoil depths at BNI, was higher compared to the deeper topsoil depths, though the trend was not significant (p > 0.10).



Figure 1.9. Aboveground biomass as a function of topsoil depth and seeding methods at BNI. Bars denote one standard error. Means with same letter are not significantly different (p<0.1). Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.

Discussion

Post-surface mining reclamation aims to reconstruct landscapes and establish diverse and productive plant communities. Newly constructed grasslands typically have homogenous soil depths and lack unique topoedaphic features that would aid in the establishment of a heterogenous plant community. Additionally, post mine landscapes may favor the establishment of exotics species which can suppress native species (Bohrer et al. 2017a). In an effort to restrict exotic grass establishment and increase potential diversity in post-mine landscapes, we investigated the effect of four topsoil depths and two seeding methods on plant community composition and biomass on two reclaimed grasslands. We found significant differences in plant composition and biomass between seeding methods and topsoil depth, but the effects differed between locations. Counter to our hypothesis, seeding method most consistently affected vegetation responses while topsoil depth's influence on vegetation responses was variable and context specific.

Our findings are not consistent with earlier studies where variable topsoil depths regularly had an effect on plant community metrics (Redente, E.F. & Hargis 1985; Redente et al. 1997; Bowen et al. 2002; Buchanan et al. 2005). However, motivations driving these studies varied, resulting in different additional main effects alongside topsoil depths. Our emphasis was on suppressing the establishment of KBG and increasing species heterogeneity. Differing conclusions could be attributed to the various research goals that address common challenges associated with reclaimed lands. Many of the previous studies that found topsoil to be the main driver of plant composition investigated the long-term impacts of treatment effects (Redente et al. 1997; Bowen et al. 2002; Buchanan et al. 2005). Topsoil depths' significant effect could likely be attributed to those additional years to decades worth of establishment time. The significant effect of topsoil depth on plant community metrics at BNI, which had one to two additional growing seasons compared to COT, suggests that time may be necessary for topsoil depths to express an effect. Time is an essential factor contributing to the interactions between abiotic and biotic components of an ecosystem which may have aided in the improvement of growing conditions.

Drivers of plant community composition varied between seeding method and location but were generally representative of grasslands in early stages of reclamation. Plant communities at BNI, seeded directly to native had a high representation of native species. These treatments had been established the longest (since June 2018) and likely were influenced by the early establishment and later recruitment of native species over multiple growing seasons. Both native and exotic species drove primary drivers of plant composition on cover crop to native sites at both locations. These sites expressed a higher species richness compared to treatments planted directly to native. Success of initial reclamation efforts in establishing native species, and

susceptibility to invasion by exotics caused by disturbance are two factors likely promoting a greater number of species (Hobbs & Huenneke 1992). Other restoration research found similar trends in that species richness tends to be highest in the early stages of restoration (Foster & Tilman 2000; Sluis 2002; Middleton et al. 2010).

Heavy representation of native, perennial C4 grass species in both seed mixes is based on the region's native grassland species composition. Species composition of sites planted directly to native at BNI had a stronger association with perennial C4 grasses than those planted with cover crop to native. However, at COT cover crop to native seeding method showed a greater association with perennial C4 grasses. The association with native, perennial, and C4 grass species for sites shows the early success of reclamation efforts and is indicative of other recently restored grasslands (Yurkonis et al. 2010). Additionally, the seasonality of species may explain the variability in species composition between seeding methods, especially when comparing the longer established sites at BNI. Sites more recently disturbed (i.e., cover crop to native) support species with a wider variety of shorter-lived species including annuals, biennials, annual/biennials, and short-lived perennials. Strong representation of short-lived species on recently disturbed ecosystems is a common occurrence (Foster & Tilman 2000; Alday et al. 2011).

Our findings suggest that species composition on our sites is in its early stages of recovery and will continue to be dynamic. A species composition heavily influenced by annuals and other short-lived species will likely transition into a perennial dominated community, leading to a decrease in species richness over time (Foster & Tilman 2000; Sluis 2002; Middleton et al. 2010). This transition to perennial dominated communities could favor native species or exotics, like KBG. Earlier work exploring plant communities of reclaimed mines

suggests exotic species cover will likely increase and become dominant as time progresses (Bohrer et al. 2017a). However, altering or improving maintenance protocols after reclamation efforts have established a desirable species assemblage could be vital for reversing this expected outcome.

Topsoil depth was a significant driver of plant community composition, though its effects were limited to the BNI location. Sites with deeper soils promoted different plant communities than shallower soil sites, but species composition varied by seeding method. Deeper topsoil depths had a greater association with C3 grasses while shallower topsoil depths indicated a greater association with C3 forbs, regardless of seeding method. These different associations of depths with grass species or forb species is shared amongst similar studies (Redente et al. 1997; Bowen et al. 2002, 2005). Previous work attributed these findings to how different growth forms compete against one another and the capabilities of forbs to establish and reproduce more readily on bare ground.

A distinct difference in plant community composition by depth between seeding method was C4 forbs, specifically Russian thistle (*Salsola tragus*; SATR) and kochia (*Bassia scoparia*; BASC). These species are more common in deeper topsoil depths and in sites planted from cover crop to native. Russian thistle and kochia are both exotic annual species frequently found on recently reclaimed land in the region. This makes the association between more recent sites (cover crop to native) and C4 forbs predictable. Supporting our initial hypothesis, these findings reveal that plant community development will vary across different topsoil depths. Earlier research found similar trends when investigating effects of variable topsoil depths on plant community dynamics (Redente et al. 1997; Bowen et al. 2002; Schladweiler et al. 2004). However, our analysis, unlike these studies, used a multivariate statistical approach to describe

the community. This method allowed plant communities within all treatments to be assessed simultaneously, providing an analysis of plant community dissimilarity based on topsoil depth. Most community analysis in previous research used single species or functional groups as response variables, limiting insights into the complexities of community dynamics between topsoil depths.

Early vegetation surveys suggest that native seeded grasses are inhibiting the establishment of Kentucky bluegrass at BNI. The negative correlation between KBG and native grass species may be attributed to priority effect (Drake 1991; Fukami 2015). Early promotion of native grass establishment through active reclamation created conditions where desired grass species were able to out compete KBG. Initial success in preventing KBG establishment was most obvious in sites planted directly to native and those sites with deeper topsoil depths (i.e., 23 and 30 cm) where percent canopy cover of KBG was lowest. As previously stated, the additional years of native plant recruitment likely assisted in out competing KBG on sites planted directly to native.

Contrary to our hypothesis, shallower soil revealed to have greater KBG coverage. Invasion of KBG in shallower soils could be due to deeper soils generally supporting native perennials grasses species which can outcompete KBG. Another factor contributing to KBG's establishment on shallow soils could be the result of reclamation practices. Soil horizons on reclaimed lands are re-constructed, stabilized, and graded separately often creating a compacted layer between the topsoil and subsoil horizons. This layer of compaction can limit water movement and produce ponding, in turn, increasing the soil moisture in the topsoil horizon. Kentucky bluegrass's shallow root system could be benefitting from any potential ponding that is occurring at the topsoil/ subsoil interface, which in the shallower topsoil depths would make soil

water more accessible (Peterson et al. 1979). Our findings reveal that current reclamation practices that apply deeper rather than shallower topsoil might be more advantageous for suppressing KBG and encouraging native grass species. However, the phenological advantage of KBG over natives could change this initial trajectory and potentially create a homogenous plant community over time.

Both main effects (seeding method and topsoil depth) played a role in aboveground biomass productivity, though these findings were only significant at BNI. Supporting our hypothesis, these findings revealed that deeper topsoil produced a greater amount of aboveground biomass. Our findings are consistent with previous research that investigated variable topsoil depths on reclaimed land in that deeper soils yielded significantly greater amounts of biomass compared to shallower soils (Redente et al. 1997; Bowen et al. 2002; Buchanan et al. 2005). Sites planted directly to native had the greatest amount of aboveground biomass, but these sites were established prior to the cover crop to native sites which is likely influencing these findings. Neither topsoil depth nor seeding method had an effect on belowground biomass productivity. Findings at both locations were highly variable likely due to the impact of different rooting structures, (i.e., taproots versus fibrous roots).

Conclusions

Grassland ecosystems are being permanently degraded by numerous abiotic and biotic influences, regionally and globally. This trend prompts the need for discovering practical means of reclaiming grasslands. We researched different seeding methods and variable topsoil depths to determine if either, or both, could increase species diversity of reclaimed grasslands while simultaneously suppressing KBG. Our investigation revealed that both seeding method and topsoil depth affected the establishment of early plant communities, though topsoil depth's impact was considerably more variable, especially by location. However, it is important to keep

in mind that the scope of our research was relatively short-term relative to the amount of time it takes land to recover from such extreme disturbances. Even so, our findings supplied valuable short-term insight into the plant community dynamics of reclaimed grasslands and how different reclamation practices are inhibiting (or creating) conditions for KBG establishment.

Though insights are derived from young, dynamic ecosystems they show our alternative reclamation efforts are creating desirable conditions one to two years post reclamation. These initial trajectories are promising, but supplemental practices may need to be integrated for the continued promotion of native species and suppression of KBG. Practices to enhance existing native cover through increased seeding rates, or maintenance of present native cover with additional future seeding may need to be considered to ensure continued suppression of KBG. Our findings indicate that both seeding method and variable topsoil depths can result in differing plant communities, and a landscape utilizing these methods could support a heterogenous plant community. However, deeper soils support greater amounts of native perennials grasses and, in turn, inhibit KBG establishment, which long-term may prevent the creation of a homogenous plant community. These landscapes are in their early stages of ecological recover and will continue to be dynamic. Therefore, further monitoring will be vital in understanding how these alternative reclamation practices impact the plant communities and ecological integrity moving forward.

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CHAPTER 2: NEWLY RECLAIMED GRASSLAND BENEFITS FROM PAIRING RIPPING WITH GRASS/FORB SEED MIXTURE

Abstract

Global energy production is in high demand and in response energy-based infrastructure is expanding its development into new landscapes, including grasslands. This expansion has intensive impacts on above and belowground components of grasslands which need to be addressed during reclamation to promote long-term ecological integrity. This study was conducted to ascertain how alternative reclamation practices may improve soil structure (i.e., compaction) while aiding in the creation of conditions that are conducive for both the establishment and continued growth of native grassland plant species. The grassland from which this study was conducted was reclaimed with different combinations of seeding mixtures (G (grass) or G/F (grass and forb), ripping techniques (SSR (subsoil ripping) or TSR (topsoil ripping), and the integration of mulch into the soil profile. Species composition and abundance of the vegetation community was estimated, and volumetric soil moisture and penetration resistance (PR) readings were obtained. Year, seed mixtures, ripping techniques and their interactions significantly affected community composition and species diversity. TSR and G/F treatment had a higher association with native, perennial grasses while SSR and G treatment favor more shortlived species (i.e., annual and short-lived perennials). Similar trends persisted across PR and soil moisture readings where TSR and G/F treatment were different from SSR and G (plus mulch) treatment(s) (p<0.10). Additionally, Kentucky bluegrass (KBG; *Poa* pratensis), an invasive grass of special concerns in the Northern Great Plains (NGP), experienced a 76% increase in abundance over one year and was more common in the TSR and G/F (plus mulch) treatment(s). While early in the reclamation process, results reveal TSR and G/F treatment are a promising

combination reclamation practice that can establish a native grassland community and initiate the improvement of compacted soil conditions.

Introduction

Global energy production and activities preceding production are expected to expand into new landscapes in response to the increased demand for energy (McDonald et al. 2009). Activities to create the infrastructure necessary for extraction and production are responsible for intensive above and belowground disturbances of otherwise intact ecosystems. Numerous negative consequences are associated with such activities including fragmentation (Trainor et al. 2016), habitat loss (Otto et al. 2016; Shaffer & Buhl 2016), alterations to soil structure (Wick et al. 2009; Stumpf et al. 2016), and the loss of productive landscapes (Allred et al. 2015). However, in many cases (i.e., surface mining) reclamation is required post-extraction to promote a return of productivity to the landscape (SMCRA 1977). Such requirements prevent the land from being abandoned, but also provides opportunities for some degree of compensation for the loss of unique ecosystems and ecological functions. Among the many ecosystems affected by the development of energy-based infrastructure are the grasslands of the Northern Great Plains (NGP) (Preston & Kim 2016), which is where multiple forms of non-renewable resources are being extracted, including lignite coal. Current traditional best reclamation practices on lignite coalmines result in reclaimed lands being successfully released from performance bonds after 10 years. Yet, ensuring soil structural recovery and sustaining a diverse plant community on reclaimed grasslands remains a challenge, especially as time since reclamation progresses (Bohrer et al. 2017a; Bohrer et al. 2017b).

Surface mining activities require large-scale excavation of earthen materials resulting in the complete deconstruction of ecosystems (Holl 2002; Pauletto et al. 2016). Excavation of all

existing vegetation and deconstruction of soil profiles is among the first stages of surface mining. Such activities results in extreme alterations to soil structure, specifically larger soil aggregates (Wick et al. 2009; Stumpf et al. 2016). Soil aggregates are further degraded by the vibrations during the course of transportation (McSweeney & Jansen 1984). These cumulative impacts on soil aggregates become problematic during reclamation as heavy load-bearing pressures from reclamation equipment (responsible for stabilizing and grading the newly constructed landscape) compress the degraded soil aggregates (McSweeney & Jansen 1984; Bohrer et al. 2017b). Compression of these altered aggregates creates compacted soil conditions, and such conditions have the potential to cause many obstacles when attempting to establish and sustain a desired plant community.

Connectivity of macropores within the soil matrix is essential for water infiltration, promotion nutrient of cycling, and providing plant roots accessibility to resources like nutrients, water, oxygen, and heat (Tracy et al., 2011; Stoessel et al., 2018). Soil compaction increases bulk density and penetration resistance (PR), reducing the distribution of macropores in the soil profile (Jabro et al. 2014), affecting growth of plant roots (Tardieu 1994; Unger & Kaspar 1994), the accessibility of water to plant roots (Haygarth & Ritz, 2009; Tracy et al., 2011), and the overall movement of water (Kulli et al. 2003). Plants must exert more energy to obtain water and nutrients, and if water cannot be obtained the plants become stressed. Additionally, limited infiltration and pooling may also occur at either the surface or subsurface, which impacts the availability of water and/or oxygen to plant roots (Hamza & Anderson 2004; Stoessel et al. 2018) and increase the likelihood of soil erosion (Stoessel et al. 2018). Decreased macropores can also impede the ability of plant roots to maneuver within the soil profile and altering the growth patterns (Hernandez-Ramirez et al. 2014; Beckett et al. 2017). These changes in root

development can directly influence water movement within the soil profile (Braunack 1986; Franklin et al. 2012) and inhibit aggregation of the soil (Wick et al. 2009; Stumpf et al. 2016), both of which contribute to the natural recovery of compacted soils. Finding a solution to improve root growth and water movement becomes vital during the reclamation process.

Alleviating soil compaction can be accomplished using a variety of anthropogenic methods, including mechanized disruption of soil or amending the soil with organic matter (Hamza & Anderson 2004). Tilling is one of the most common land management practices used to decrease soil compaction (Schneider et al. 2017). This technique breaks up the compressed layer of soil and increases the amount and distribution of macropores (Hangen et al. 2002). Tilling-like practices applied to reach subsoil depths is often referred to as ripping, or subsoiling (Schneider et al. 2017). An additional means of decreasing compaction is the integration of organic matter (OM), e.g. material such as straw, into the soil (Getahun et al. 2018). This management practice can aid in alleviating compaction in two ways. The capabilities of OM to absorb water improves the soil water-holding capacity enhancing the availability of water to plant roots (Zhao et al. 2014). Also, as organic materials decompose they aid in soil aggregation by adding organic carbon (Sheoran 2010). The application of these practices improves the pore space distribution which in turn promotes water movement, root exploration, and decreases the bulk density and penetration resistance. Ultimately, these actions have the potential to improve growing conditions and the establishment of desirable native grassland species. However, these conditions may also promote invasive species like Kentucky bluegrass (*Poa pratensis*), a cool season invasive grass of special concern in the NGP.

Grasslands in the NGP are being disturbed to support energy production-based infrastructure (Preston & Kim 2016), but mandatory reclamation for surface-mining operations

provides an opportunity for native grasslands to be replaced by new reconstructed grasslands. Unfortunately, conditions of older reclaimed grasslands, both above and belowground, are not presenting ecological qualities representative of functional grasslands (Bohrer et al. 2017a; Bohrer et al. 2017b), prompting a need to investigate alternative reclamation practices.

The objective of this study was to compare how different combinations of alternative reclamation practices can influence community composition, reduce PR, and improve soil water movement. The alternative reclamation practices applied include combinations of either ripping the subsoil horizon prior to topsoil replacement or ripping through the topsoil and subsoil after topsoil replacement; the integration of straw mulch within the subsoil horizon or no mulch; and one of two seed mixtures consistently of either just grass or grass and forbs. We expect to observe quantifiable differences between the different combinations when assessing the plant community composition, PR, and volumetric soil moisture. Assessment of these different combinations will help mining companies evaluate whether current traditional best reclamation practices can, or should, be adjusted to better promote the above and belowground components of reclaimed grasslands, long-term.

Methods

Site Description

This study took place on BNI Mine Ltd property, located approximately 6 km SE of Center, ND (lat. 47°02' 52.66" N long. 101°14'31.61" W). This project site falls within the Northern Great Plains ecoregion with temperatures ranging between -11°C and 22°C (NDAWN 2020), and an average of 150 frost-free days per year (USDA-NRCS 2006). Average growing season precipitation is 355 mm (NDAWN 2021). Soils consisted of silt loams, loams, and silty

clay loams complexes prior to mining; however, they are then reclassified as "mined-land complex" (USDA-NRCS 2021a) post-mining.

Experimental Design

The project site was stripped mined between 2015 and 2016, and subsequent reclamation completed by April/May of 2018. Installation of our research plots occurred during reclamation and comprised of the following factors: two seeding mixtures, deep ripping at one of two phases of reclamation, and incorporation of mulch into the subsoil horizon. All combinations of factors are represented at the designated location.

Half of the treatments were planted to a grass only seed mixture (G) and the other half seeded to a grass and forb seed mixture (G/F) (Table 2.1). Deep ripping occurred either within the subsoil horizon prior to topsoil replacement (SSR) or across both the subsoil and topsoil horizons after topsoil replacement (TSR). Ripping shanks reached maximum depths of 56 cm. Straw mulch was applied to the subsoil surface at 1130-1360 kg/ha and then incorporated into the surface of the soil via disking. The reference site was seeded with the grass/forb mix and proceeded with standard reclamation practices which did not include ripping or mulch. Each combination was replicated twice and the reference site once (Figure 2.1), and each unit was approximately 0.6 hectares.



Figure 2.1. BNI Ltd project design layout. Reclamation combinations consist of a grass only seed mixtures (G) or a grass and forb seed mixture (G/F); the use of ripping at the subsoil horizon prior to topsoil replacement (SSR) or the use of ripping from the topsoil horizon after topsoil replacement (TSR); integration of straw mulch into the subsoil horizon prior to topsoil replacement.

Common Name	Scientific Name	Growth Form	kg pls/ha	% of Seed Mix
Oats	Avena spp.	Graminoid	1.79	22.86
Big Bluestem	Andropogon gerardi	Graminoid	0.56	7.15
Blue Grama	Bouteloua gracilis	Graminoid	0.44	5.62
Green Needlegrass	Nassella viridula	Graminoid	2.69	34.36
Little Bluestem	Schizachyrium scoparium	Graminoid	0.67	8.56
Sideoats Grama	Bouteloua curtipendula	Graminoid	0.45	5.75
Switchgrass	Panicum virgatum	Graminoid	0.67	8.56
Western Wheatgrass	Pascopyrum smithii	Graminoid	0.56	7.15
Blanketflower	Gaillardia aristata	Forb*	0.11-0.36	
Hoary Vervain	Verbena stricta	Forb*	0.11-0.36	
Purple Prairie Clover	Dalea purpurea	Forb*	0.11-0.36	
Stiff Goldenrod	Solidago rigida	Forb*	0.11-0.36	
Wild Bergamot	Monarda fistulosa	Forb*	0.11-0.36	
Black-eye Susan	Rudbeckia hirta	Forb*	0.11-0.36	
Purple Cone Flower	Echinacea purpurea	Forb*	0.11-0.36	

Table 2.1. List of all species and amount of each species used in BNI seed mix. Includes both common and scientific names. The cover crop was an annual oat species (*Avena* spp.).

* A minimum of 3 species are chosen among this list each year, depending on availability. Forbs account for 5% of the total seed mix.

Data Collection

Vegetation Data

Plant community composition and canopy cover surveys were conducted by randomly establishing three 60-meter transects in each treatment unit. We placed a 0.5 m^2 frame every 15-meters along each transect and identified every plant to a species level. We measured abundance by estimated canopy cover of each species and assigned one of the following modified Daubenmire cover classes, 1= trace -1%, 2= 1-2%, 3= 2-5%, 4= 5-10%, 5= 10-20%, 6= 20-30%...13= 90-95\%, 14= 95-98\%, 15= 98-99\%, and 16= 99-100\% (Daubenmire, 1959).

Surveys were conducted in 2019 and 2020 during peak production (mid-July) and midpoint values were used for analysis. We referenced the USDA Plant Database (USDA-NRCS 2021b) and Minnesota Wildflowers (MN Wildflower 2021) websites to classify each species' seasonality, metabolic pathway, and origin (native versus exotic) after completion of surveys.

Soil Moisture Data

We installed three soil moisture access tubes into the soil profile of each treatment unit. Tubes were a minimum of 30-meters in from the plot's edges, a minimum of five-meters from other units and installed to a depth of one meter. A soil moisture probe was used to take three separate readings at 10, 20, 30, 40, 60, and 100 cm intervals (Delta-T Device Ltd, UK). We rotated the probe 120° before the second and third readings to account for any soil moisture variability within the soil profile. Our analysis used averages from each tube.

Soil Penetration Resistance Data

Four penetration resistance readings were taken to a depth of one meter with an automated dynamic cone penetrometer (ADCP; Vertek, USA). We obtained each reading by positioning the ADCP approximately three meters away from the associated access tubes in each of the cardinal directions. Readings automatically recorded were later converted to joules per meter. The method for calculating penetration resistance is based on the soil's capacity to cease work being performed by the penetrometer divided by how far the penetrometer progressed:

$$R_s = \frac{W_s}{P_d} \tag{1}$$

Where: R_s is the soil resistance (N), W_s is the kinetic energy of the (J), and P_d is the depth traveled by the penetrometer through the soil (Equation 1).

Statistical Analysis

Vegetation Dynamics

We used averaged species cover values calculated from all frames within a transect across each treatment replication. Generalized linear models were used to test the effects of seeding method, ripping technique, and mulching and their interactions on species richness, diversity, and KBG cover. Tukey post-hoc tests followed any significant ANOVA results ($p\leq$

0.10). We performed a separate analysis for each year of data for models of species richness, diversity and KBG cover. We ran nonmetric dimensional scaling analyses (NMDS) and permutational multivariate analysis of variance (PERMANOVA) for 2019 and 2020 separately to understand dissimilarities in species composition between each treatment (Oksanen et al. 2019). Ordinations were calculated in three dimensions using Bray-Curtis distance measures. To further assess the differences in community composition we added plant functional trait data as vectors using *envfit*. We performed all our analyses using R version 4.0.4 (R Development Core Team 2021) and the following packages car (Fox & Weisberg 2019), agricolea (Mendiburu 2020), vegan (Oksanen et al. 2019).

Soil Moisture

We used linear mixed effect models to analyze each of the soil moisture depth intervals. We included treatment as the fixed effect and year, replication, and observation point random effects. Tukey's post- hoc tests were performed for those soil moisture depth intervals that returned significant differences between treatments ($p \le 0.10$). We used lme4 (Bates et al. 2015) for our linear mixed effect model and lsmeans (Lenth 2016) for our post- hoc tests. These same packages were used in the following section as well.

Penetration Resistance

We selected three separate depth bins of 0-15, 15-30, and 30-100 cm after a preliminary analysis determined no significant differences for depth intervals falling between 30 and 100 cm. We averaged the four ADCP readings at each observation point to simplify our model random effects structure. We found no interaction between treatment and depth allowing treatment to be a fixed effect in our linear mixed effect model. Volumetric soil moisture readings were included as a covariate to our models to account for the variability of soil moisture at different depths

which may influence PR values. We used the 10, 20, and 60 cm soil moisture depth interval averages for the 0-15, 15-30, and 30-100 cm depth bins, respectively. Readings from the ADCP took place in the same location over the course of three years. Therefore, we included year, replication, and observation point (i.e., location associated with the access tube) as nested random effects in our model. We ran this linear mixed effect model separately for all three depths. We performed subsequent Tukey's post-hoc procedures with 90% confidence intervals when the model returned significant differences between treatments ($p \le 0.10$).

Results

Species Richness and Diversity

Our surveys resulted in the identification of 52 and 57 species in 2019 and 2020, respectively. Seven grass species and five forb species present in the seed mix were found in both 2019 and 2020. Both ripping, seeding mixture, and a ripping/seeding interaction showed a significant effect on species richness in 2019 ($p\leq0.10$) (Figure 2.2). Species richness ranged between an average of 18 and 9 species in 2019, and 16 and 9 species in 2020. However, only seeding mixture influenced species richness in 2020 ($p\leq0.10$) (Figure 2.3). Treatments planted to G/F had significantly more species in both years, while treatments reclaimed with TSR had significantly more species only in 2019. Simpson diversity showed a wider range of values between treatments in 2019 compared to 2020 with values ranging from 0.75-0.47 and 0.84-0.75, respectively (Figure 2.4 and 2.5). Trends show that treatments reclaimed with TSR and treatments planted with G/F have the largest average diversity values in 2019 and 2020, respectively. Mulch did not have any significant impact on either species richness or diversity.



Figure 2.2. 2019 plant species richness as function of reclamation treatments (A), seeding mixture (B), and ripping technique (C). Bars denote one standard error. Means with same letter are not significantly different ($p \le 0.10$). Dotted line represents mean value of reference site. Data were collected in July 2019 from BNI Coal, LTD, near Center, ND USA.



Figure 2.3. 2020 plant species richness as function of reclamation treatments. Bars denote one standard error. Means with same letter are not significantly different ($p \le 0.10$). Dotted line represents mean value of reference site. Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.



Figure 2.4. 2019 plant species diversity as function of reclamation treatments. Bars denote one standard error. Dotted line represents mean value of reference site. Data were collected in July 2019 from BNI Coal, LTD, near Center, ND USA.



Figure 2.5. 2020 plant species diversity as function of reclamation treatments. Bars denote one standard error. Means with same letter are not significantly different ($p \le 0.10$). Dotted line represents mean value of reference site. Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.

Species Composition and Abundance

Year (p=0.001) (Figure 2.6) and two of the main effects, seeding (p=0.002) and ripping (p=0.006), impacted plant community composition, when both years were assessed collectively. Additionally, year and seeding (p= 0.037) and ripping and seeding (p=0.022) interactions influenced species composition and abundance. Consequently, subsequent analyses were separated by year to evaluate how the main effects impacted the plant community. We used functional groups to explain primary drivers of plant composition.



Figure 2.6. NMDS ordination of plant community composition by year. Species composition of treatments with ellipses representing years; k=3, stress= 0.19. Data were collected in July 2019 and 2020 from BNI Coal, LTD, near Center, ND USA.



Figure 2.7. 2019 NMDS ordination of plant community composition. Species composition of 2019 with ellipses representing seeding mixtures (solid lines) and ripping technique (dashed lines); k=3, stress= 0.20. Vectors provide community composition associations given different metabolic/life-forms. Vectors not labelled were not significant. Species code coloration is dictated by whether the species was in the seed bank (i.e., Species Not Seeded; black text) or part of the seed mixes (i.e., Species Seeded; grey text). Data were collected in July 2019 from BNI Coal, LTD, near Center, ND USA.

Both seeding and ripping, and a seeding/ripping (p=0.056) interaction had a significant

effect on the plant community composition in 2019 ($p\leq 0.10$) (Figure 2.7). The primary

functional groups driving the 2019 plant community composition were native, perennial, C3

grasses on NMDS axis 1, short-lived perennials on NMDS axis 2, and annual/biennials and C3

forbs on NMDS axis 3 ($p\leq0.10$) (Table 2.2). Additionally, percent volumetric soil moisture at 30

and 40 cm depth ranges, around the topsoil/subsoil interface were primary drivers of plant

composition on the NMDS 2 and NMDS 1, respectively (Table 2.2).

Table 2.2. 2019 Functional group correlation coefficients. Correlation coefficients for NMDS 1, NMDS 2, NMDS 3. Bold values indicate whether a functional group or soil moisture had significant influence in explaining the 2019 plant community. Data were collected in July 2019 from BNI Coal, LTD, near Center, ND USA.

Functional Group Category & Soil Moisture	NMDS 1	NMDS 2	NMSD 3	r^2	Pr(<r)< th=""></r)<>
C3 Grass Species	0.92664	0.37594	0.00339	0.3165	0.001
C4 Grass Species	-0.17913	0.37266	0.91052	0.1026	0.173
C3 Forb Species	0.35647	0.59547	0.71996	0.3845	0.001
C4 Grass Species	-0.33139	0.03425	-0.94287	0.0675	0.369
Annual Species	-0.25252	-0.02744	0.9672	0.0467	0.496
Perennial Species	0.86407	-0.49478	-0.09255	0.4502	0.001
Short-lived Perennial Species	-0.34945	-0.75169	-0.55933	0.39	0.001
Annual/Biennial Species	-0.52172	-0.12435	-0.844	0.3712	0.001
Biennial Species	0.61813	-0.19989	-0.76024	0.1136	0.123
Native Species	0.89503	0.2887	0.33995	0.4726	0.001
Exotic Species	-0.4772	0.87619	0.06754	0.1324	0.079
Vol. Soil Moisture at 30 cm	-0.43898	0.78042	0.44524	0.236	0.003
Vol. Soil Moisture at 40 cm		0.69028	0.60553	0.39605	0.3864

Seeding and ripping were significant main effects in 2020 ($p\leq0.10$), and additional analysis revealed a significant seeding/ripping interaction (p=0.016) (Figure 2.8). Species composition in 2020 was primarily driven by C4 species, and annuals on NMDS axis 1, long and short-lived perennials and native species on NMDS axis 2, and all C3 species on NMDS axis 3 ($p\leq0.10$) (Table 2.3). Both 30 and 40 cm depth ranges for percent volumetric soil moisture were also a primary drivers of species composition on NMDS axis 2 (Table 2.3).



Figure 2.8. 2020 NMDS ordination of plant community composition. Species composition of 2020 with ellipses representing seeding mixtures (solid lines) and ripping technique (dashed lines); k=3, stress= 0.18. Vectors provide community composition associations given different metabolic/life-forms. Vectors not labelled were not significant. Species code coloration is dictated by whether the species was in the seed bank (i.e., Species Not Seeded; black text) or part of the seed mixes (i.e., Species Seeded; grey text). Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.

Table 2.3. 2020 Functional group correlation coefficients. Correlation coefficients for NMDS 1, NMDS 2, NMDS 3. Bold values indicate whether a functional group or soil moisture had significant influence in explaining the 2020 plant community. Data were collected in July 2020 from BNI Coal, LTD, near Center, ND USA.

Functional Group Category & Soil Moisture	NMDS 1	NMDS 2	NMSD 3	r ²	Pr(<r)< th=""></r)<>
C3 Grass Species	0.07433	0.699	-0.71125	0.2241	0.008
C4 Grass Species	0.80267	0.44474	0.39741	0.6557	0.001
C3 Forb Species	0.49553	-0.57974	0.6468	0.2424	0.004
C4 Grass Species	-0.95969	0.08085	0.26919	0.3398	0.001
C3 Shrub Species	-0.14691	-0.27682	-0.94963	0.1147	0.091
Annual Species	-0.9541	-0.0225	0.29865	0.3279	0.001
Perennial Species	0.56804	0.80983	-0.14665	0.379	0.001
Short-lived Perennial Species	0.02459	-0.99898	-0.03787	0.4108	0.001
Annual/Biennial Species	-0.04414	-0.99613	0.07596	0.0979	0.195
Biennial Species	0.75277	-0.30266	-0.58458	0.0545	0.486
Native Species	0.52074	0.8537	-0.00552	0.2417	0.005
Exotic Species	-0.77477	-0.62804	-0.07285	0.094	0.186
Vol. Soil Moisture at 30 cm	-0.21805	-0.92383	0.314622	0.1822	0.021
Vol. Soil Moisture at 40 cm	-0.17947	-0.97361	0.140984	0.1448	0.053

Year influenced Kentucky bluegrass (*Poa pratensis*) abundance (p=0.029) with percent cover increasing by 76% between 2019 and 2020 (Figure 2.9). Seeding mixture (p=0.017) and ripping (p=0.004) influenced the abundance of KBG when both years were assessed collectively (Figure 2.8). Site(s) planted with G and reclaimed with SSR (plus mulch) had significantly less KBG compared to the site planted to G/F and reclaimed with TSR (Figure 2.9).



Figure 2.9. Percent canopy cover of Kentucky bluegrass (*Poa pratensis*). Percent cover as a function of year (A) and treatment with both years assessed collectively (B) at BNI. Bars denote one standard error. Means with same letter are not significantly different ($p \le 0.10$). Data were collected in July 2019 and 2020 from BNI Coal, LTD, near Center, ND USA.

Penetration Resistance

Early trends indicate that treatment(s) reclaimed with SSR and planted with G (plus mulch) have the highest PR values with means of 35.9 (37.1), 44.9 (50.2), and 45.7 (48.7) J/m; respective, to treatment(s) and depths. Furthermore, those treatments planted with G/F and reclaimed with TSR consistently have the lowest PR values with means of 25.6, 28.1, and 33.2 J/m; respective by depth. The treatments with the highest mean PR readings are significantly different than treatments with lowest mean PR readings, at all three depths ($p \le 0.10$) (Figure 2.10). G, SSR, plus mulch treatments at the 15-30 cm depth bin were statistically different from all other treatments other than the G and SSR treatment. Additional differences between

treatments exist at depths of 15-30 and 30-100, but differences in means appear to be between those with the same seeding mixture or the same ripping technique. There was no indication that standard reclamation procedures (i.e., the control) resulted in significantly different penetration resistance readings at any depth, at this time (Figure 2.10).



Figure 2.10. Penetration resistance by depth as a function of reclamation treatments. Three depth bins were 0-15, 15-30, and 30-100 cm. Values are in Joules per meter. Bars denote 90% confidence intervals. Dotted line represents mean value of reference site. Data were collected in between July and September in 2018 -2020 from BNI Coal, LTD, near Center, ND USA.

Volumetric Soil Moisture

Treatments planted with G, SSR, plus mulch were significantly different to treatments planted with G, TSR, plus mulch and treatments planted with G/F and TSR ($p\leq0.10$), at the 20, 30, and 40 depth intervals. Additionally, trends reveal there are significant differences between treatments with the same seed mixtures and/or ripping techniques at both 30 and 40 cm depth

intervals. Treatment(s) planted with G/F and reclaimed with TSR (plus mulch) were most frequently different to those treatments across both seeding mixtures and ripping techniques (Figure 2.10).

Standard reclamation procedures (i.e., reference site) had significantly greater (p<0.10) volumetric soil moisture at the 40 cm depth than those treatments planted with G/F and reclaimed with TSR. Volumetric soil moisture readings at the 10 and 60 cm depths showed no differences between treatments (p>0.10) (Figure 2.11). We did not assess the 100 cm depth interval because it likely has no influence over plant community dynamics this early in the reclamation phase. At the 20, 30, and 40 depth intervals mean percent values of those treatments planted with G, SSR, plus mulch (22.3, 23.7, 26.0, respective of depth) had significantly greater volumetric soil moisture compared to treatments planted with G, TSR, plus mulch (16.7, 18.8, 19.4, respective of depth) and treatments planted with G/F and TSR (17.2, 17.9, 15.7, respective of depth). The number of treatments showing significant differences increased with depth with the greatest variability being observed at both the 30 and 40 cm depth intervals. Significant differences between combinations exist between treatments with the same ripping techniques and same seeding mixture ($p \le 0.10$) (Figure 2.11). Treatment(s) planted with G/F and reclaimed with TSR (plus mulch) were most frequently different to those treatments across both seeding mixtures and ripping techniques (Figure 2.11). Standard reclamation procedures (i.e., the reference site) had significantly greater volumetric soil moisture at the 40 cm depth than those treatments planted with G/F and reclaimed with TSR (p<0.10). Volumetric soil moisture readings at the 10 and 60 cm depths showed no differences between treatments (p>0.10) (Figure 2.11).



Figure 2.11. Volumetric soil moisture by depth as a function of reclamation treatments. Three separate depths were 20, 30, and 40 cm. Values as percentages. Bars denote 90% confidence intervals. Dotted line represents mean value of reference site. Data were collected in between July and September in 2018 -2020 from BNI Coal, LTD, near Center, ND USA.

Discussion

Pre-extraction processes of surface mining and subsequent reclamation activities provide efficient means of de-constructing and re-constructing a new landscape. However, belowground conditions resulting from these current best management practices (i.e., compacted soils) produce challenging circumstances for establishing and sustaining a new diverse plant community (Bohrer et al. 2017a). Furthermore, the presence and establishment of exotic species, like KBG, add additional stresses to fostering a desirable plant community (Bohrer et al. 2017b). In an attempt to address these various challenges associated with a reclaimed landscape, we explored the impacts of alternative reclamation practices on a newly reclaimed grassland. We found that plant composition significantly differed between 2019 and 2020, and two out of three of the treatment effects (i.e., seed mix and ripping) had a significant effect on the plant community composition, regardless of year. Additionally, penetration resistance and soil moisture readings are showing early trends that suggests a distinction between treatments.

Distinct plant community assemblages are beginning to form in response to seeding mixtures, ripping techniques, and their interaction over time. TSR and G/F treatments frequently shared plant community primary drivers across all functional groups, in 2019. These treatments were commonly associated with native perennials, C3 grasses, and C3 forbs. This trend is likely attributed in large part to the seed mix, but also the improved growing conditions for vegetation provided by ripping from the topsoil horizon (Ashby 1997; Bauman et al. 2014; Fields-Johnson et al. 2014). C3 grasses occurred more often with G/F and TSR treatments, yet planted C3 grass species (i.e., NAVI (*Nassella viridula*) and PASM (*Pascopyrum smithii*)) were commonly found on all treatments in both years. The USDA Natural Resources Conservation Service recommended these native species for revegetating disturbed/reclaimed landscapes which likely contributes to their high proportion use in the seed mix and them being well represented.

Generally, shorter-lived species (i.e., annuals and short-lived perennials) occurred in G and SSR treatments in 2019. This representation of short-lived species is typical of recently disturbed/reclaimed landscapes (Foster & Tilman 2000; Alday et al. 2011) and may explain some of the increases of species richness found among G and SSR treatments experienced from 2019 to 2020. However, some of the G/F and TSR treatments also experienced increases in species richness between 2019 and 2020 without a heavy association with short-lived species. This

fluctuation of species richness in the early stages of ecological recovery is a commonly documented occurrence in previous grassland restoration work (Sluis 2002; Middleton et al. 2010). These changes in species richness between 2019 and 2020 suggests that all treatments, but especially G and SSR treatments, may experience more pronounced shifts in species composition over time.

By the second sampling season interactions between TSR and G/F treatments and G and SSR treatments became increasingly more noticeable. However, community assemblages between seeding mixtures were found to be less distinct from one another which expresses the dynamics of recently reclaimed grasslands. For example, while intentionally seeded C3 forbs occurred more frequently in G/F and TSF treatments, C3 forbs were a primary driver of the vegetation community for G and SSR treatments. The expression of those short-lived species found on G and SSR treatments in 2019 and the retention of longer lived C3 forb species found in G/F and TSR treatments may have prompted this shift in assemblages between seeding mixtures. Additionally, C4 grasses and C4 forbs (i.e., SATR (Salsola tragus) and BASC (Bassia scoparia); annual invasive species) became prominent drivers of species composition in 2020. The C4 grasses appear to more readily occupy G/F and TSR treatments, but C4 forbs seemingly occupy all treatments. This C4 grass prominence is not wholly uncommon in restored grasslands (Camill et al. 2004), but the broad occurrence of C4 forbs across all treatments may contribute to the increased similarity between seeding mixtures' community assemblages. Early predominance of seeded species, especially the forbs and C4 grasses, indicates that G/F and TSF treatments are promoting conditions for a desirable plant community, with the exception of KBG. Yet, the interaction between G/F and TSR and G and SSR treatments, along with the overlapping

community composition that exists among these different main effects may indicate that these communities are becoming increasingly more similar over time.

One notable trend not observed on any of our treatments was the strong influence of exotic species. This is especially notable given previous research efforts attributed uneven surfaces created by ripping/disking/tilling to increased weed production via seed capture (Redente, E.F. & Hargis 1985). In the case of our study, treatments that experienced TSR (i.e., surface level disturbance) had a greater association with natives in 2019 and 2020. Strong establishment of native species from the seed mixtures and/or seed bank may be inhibiting the initial establishment of exotic species, preventing exotic species to be dominate drivers of species composition. However, chances of invasion by aggressive non-native species can, and often do, increase over time.

Early findings suggest, when used together, TSR and G/F mixtures could aid in improving the growing conditions, especially for native, perennial, C3 grasses and intentionally seeded C3 forbs species. However, our results found that those treatments actively reclaimed with combinations of alternative reclamation practices were not significantly different to standard practices (i.e., the reference site). Thus, early trends indicate that standard practices are providing relatively similar conditions to those created by alternative reclamation practices. One explanation for this trend could be attributed to the study design. Unlike all the other treatments the reference site only had one sampling plot resulting in half the number of samples. This uneven number of sampling points could affect the accuracy of our findings with regards to comparing the reference site to those alternative reclamation practices. It must be noted though that while TSR and G/F were found to have the lowest PR values, average PR values at all depths for these treatments fell between 4.25-6.13 MPa, which is well above the 2 MPa that

restrict root penetration (Benjamin et al. 2003). Additionally, the application of ripping occurred only once in this study and settling and dispersion of soil particles due to rain events often result in re-compaction of previously ripped/tilled areas (Busscher et al. 2002). For this reason, it is important that PR readings continue to be taken to determine if the effects of these alternative reclamation practices will persist or change over time.

Variability between reclamation combinations was most notable at the 30 and 40 cm depth intervals which is most likely the depth where ripping activities, from the 56 cm ripping shanks, were most impactful for those TSR treatments. Generally, at these depths TSR treatments had the lowest volumetric soil moisture compared to those that received SSR. Decreased soil moisture on TSR treatments at these depths indicates greater infiltration and dispersion of water is occurring likely as a function of the mechanical creation of macropores (Hangen et al. 2002). Comparatively, SSR which experienced no surface-level ripping activities, had greater volumetric soil moisture possibly attributing to the disproportionate amount of micropores that developed from compacted conditions that developed during pre and post mining activities. Given smaller pores tend to hold water more tightly, perhaps the micropores contributed to a greater retention of water. While surface-level manipulations, or the lack of, seems to be the simplest explanation for these trends, variation of volumetric soil moisture between treatments was also associated, to varying degrees, with the seed mixtures. Treatments with combinations of TSR and G/F consistently showed significant differences from SSR and G plus mulch treatments, as with PR and community composition. However, the TSR treatments are still relatively compacted, and without species specific root data it is hard to determine how much the individual species are influencing the soil moisture at the 30 and 40 cm depth intervals.

Conclusions

Current traditional best reclamation practices struggle to establish and sustain a native landscape due to challenges often associated with soil compaction and aggressive non-natives. Alleviating soil compaction due to surface mining often times takes place years to decades after reclamation had ceased and/or frequently emphasizes the establishment of woody plant communities. We investigated how combinations of alternative reclamation practices, applied during the process, affected native grassland species composition, and influenced KBG establishment. Generally, our findings revealed when G/F seed mixtures are combined with TSR (plus mulch) intentionally planted native species will be well represented and PR resistance will be lower compared to other combinations of reclamation practices. Unfortunately, KBG abundance was also most closely associated with these treatments, which complicates the otherwise promising projections for combating soil compaction and promoting a greater diversity of native grassland species. Those treatment(s) planted to G and reclaimed with SSR (plus mulch) had higher PR at all depths and were not as strongly associated with planted native species, but these treatment(s) had less KBG cover compared to other reclamation combinations.

These findings provide valuable insight into early stages of ecological recovery for reclaimed grasslands as a function of these alternative reclamation practices. However, it is important to note that PR values for the reference site (i.e., standard practices), at all three depths, were not different from any of the alternative reclamation practices. Yet, ecological recovery of newly reclaimed landscapes takes time, and as time progresses some of the beneficial conditions created by our alternative practices could be enhanced or regress, changing the current trajectory. Therefore, continued monitoring of these difference reclamation

combinations is important for understanding the effects soil properties have on reclaimed grasslands plant communities to determine if intermittent maintenance is necessary long-term.

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