RECOVERY OF RECLAIMED SOIL STRUCTURE AND FUNCTION IN RELATION TO PLANT COMMUNITY COMPOSITION[1]

A.F. Wick[2], P.D. Stahl, S. Rana, and L.J. Ingram

Abstract: Recovery of belowground properties, such as soil aggregation and microbial community composition, in relation to aboveground plant community characteristics is important for determination of reclamation success. The objectives of our research were: (1) to track ecosystem recovery based on soil aggregation and microbial biomass in different reclaimed soils (sandy loam and clay loam) and under different plant communities (shrub and cool season grass) through time and (2) to find relationships among aboveground plant composition, soil aggregate size distribution and microbial biomass. We hypothesized that the belowground properties of soil aggregation and microbial communities will recover simultaneously through time and will be highly related to the aboveground plant community composition. Aggregate size distribution did not show any patterns in the sandy loam soils underlying shrub communities; however did show progress towards a native condition in the clay loam soils underlying the cool season grass communities. Soil microorganisms showed recovery after 5 years (29.6×10^3 area) in soil underlying shrub communities and after 14 years (22.0×10^3 area) in soils underlying cool season grass communities. Macroaggregates were related to bacteria (r = 0.40) and fungal (r = -0.31) biomass under cool season grasses. Microaggregates were correlated with bacteria biomass under both shrub (r = -0.26) and cool season grass (r = -0.40) communities and also to living fungal biomass (r = -0.39) in the shrub soils. Microaggregation, fungal and actinomycete biomass were correlated in native cool season grasses (r = -0.25, 0.66 and -0.47, respectively) and annual forbs (r = 0.52, -0.75 and 0.49, respectively) in shrub community soils. Macroaggregates, microaggregates and bacteria were related to native cool season grasses (r = -0.61, 0.56 and -0.39, respectively), annual grasses (r = 0.69, 0.71 and 0.37, respectively) and annual forbs (r = 0.69, -0.40 and 0.41, respectively). The ability to track aggregate and microbial changes through time as well as find relationships amongst ecosystem variables leads to better reclamation techniques and success.

Additional Key Words: coal mine, phospholipid fatty acids, Wyoming.

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[2]Abbey F. Wick, Graduate Student, Peter D. Stahl, Assistant Professor, Sadikshya Rana, Graduate Student, and Lachlan J. Ingram, Post-Doctoral Research Associate, respectively, University of Wyoming, Department of Renewable Resources, Laramie, WY 82071, (307) 766-2263.

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Introduction

In order to determine the recovery of reclaimed ecosystems, defined as the progression of reclaimed systems towards native systems, the relationships between physical and biological properties of soils as well as vegetation characteristics need to be understood. Inadequate recovery of soil physical and biological properties in reclaimed soils can lead to future site degradation and vegetation failure (National Research Council, 1994). In both reclaimed and native soils, there are important relationships among soil aggregation, microbial activity, above- and belowground plant community composition, soil organic matter (OM) content and nutrient cycling, soil texture, salts, soil stability and climate parameters which influence ecosystem recovery and stability (Alexander and Middleton, 1952; Jastrow, 1987; Jastrow et al., 1998; Kay, 1998; Vogel, 1987; Six et al., 2002; Valmis et al., 2005).

Highly structured soils contain various sized aggregates which physically protect OM from microbial decomposition. This protection mechanism results in reduced OM turnover rates and a steady release of plant available nutrients compared to soils lacking aggregation. With disturbance, macroaggregates (>250 μm), held together primarily by roots and fungal hyphae, break apart and release microaggregates (53-250 μm) as well as silt and clay particles (<53 μm) into the soil matrix making once protected OM susceptible to higher rates of microbial decomposition (Tisdall and Oades, 1982; Six et al., 1998). A high proportion of microaggregates compared to macroaggregates is characteristic of disturbed soils (Six et al., 1998). Following reclamation, microbial polysaccharides, root exudates and OM are added to the soil as roots and fungal hyphae re-develop. Macroaggregates start to form from recent and old OM, free microaggregates, silt and clay (Six et al., 1998; Tisdall, 1996). Once stable macroaggregates are formed, microaggregates will develop within the macroaggregates through the binding of complex OM, silt and clay (a concept known as aggregate hierarchy, Tisdall and Oades, 1982). This type of microaggregate is extremely stable due to enhanced protection within macroaggregates. They have a lower porosity compared with macroaggregates and provide a mechanism for long term carbon (C) storage (Oades, 1993; Six et al., 2002). In addition to nutrient cycling and C dynamics, soil aggregation also increases soil pore space (as indicated by a reduction in bulk density), pore size distribution and reduces soil erosion (Vogel, 1987). For these reasons, structural recovery of soil in drastically disturbed lands is important for ecosystem function and reclamation success (Vogel, 1987).

Another vital component to reclamation success is soil microbial community structure, which also influences nutrient cycling and C dynamics in soils. In recently disturbed soils, bacteria communities thrive and dominate nutrient cycling (Visser et al., 1983; Moore and de Ruiter, 1991; Lovell et al., 1995). As soils recover after disturbance, fungal communities recover and contribute to nutrient cycling, C dynamics, and plant water uptake (Cambardella and Elliott, 1994; Stahl et al., 1999; Allison et al., 2005). The recovery of fungi also contributes to water uptake by vegetation. Certain species of fungi (mutualistic) form symbiotic relationships with plants and act as root extensions to aide in water uptake during drought conditions (Paul and Clark, 1996). This, in turn, affects aggregate formation and stability (Six et al., 2004). With the recovery of microbial communities and associated C storage and nutrient cycling, reclaimed ecosystems can be restored to pre-disturbance levels (Vogel, 1987).

Plant community composition and net primary production, both above- and belowground, also influence soil and ecosystem recovery. Plant influences vary based on root structure, water use efficiency and rhizodeposition (Rillig and Mummey, in press). Differences in root thickness and root physical force greatly affect aggregate formation and fungal associations (Jastrow et al., 1998; Six et al., 2004). Aggregates usually recover more quickly under annual and perennial forbs and are more stable (Vogel, 1987). Drying and wetting of the soil from plant uptake of soil water increases aggregate stability (Six et al., 2004). Revegetation species with higher productivity release more carbohydrates into the soil (rhizodeposition), which drives microbial activity and indirectly aggregate formation (Vogel, 1987; Paul and Clark, 1996). In addition to vegetation contributions while living, plants contribute to soil processes upon death. More than 50% of root biomass senesces annually, and along with surface litter, contributes OM to the soil matrix (Paul and Clark, 1996).

The objectives in this study were to investigate relationships between and track changes in soil aggregation, microbial and reestablished plant community biomass in different site ages to gain a better understanding of reclaimed soil dynamics. We hypothesize that (1) as soil aggregation recovers, microbial communities will also recover and (2) plant community composition will be related to soil aggregation and microbial communities.

Methods and Materials

Site Information

Chronosequences (substitution of space for time) were used to study the recovery of soil aggregation, microbial communities, vegetation community dynamics and the relationship between these variables through time. Though spatial variability within each site in the chronosequence can be greater than temporal variability represented by the chronosequence; use of this technique is necessary for the evaluation of reclamation treatments. Utilizing chronosequences can help understand change between soil and plant community relationships due to the long duration required to determine reclamation success, particularly in semi-arid ecosystems. With this in mind, sites were selected with similar plant communities (shrub or cool season grass), topography (<1% slope), soils and climate at a single mine.

Two chronosequences of reclaimed sites were sampled at the Dave Johnston and Belle Ayr Mines in the Powder River Basin, WY, USA (Fig. 1). The shrub chronosequence consisted of 5 sites which included <1, 5, 10, and 16 year old reclamation and an undisturbed site with native

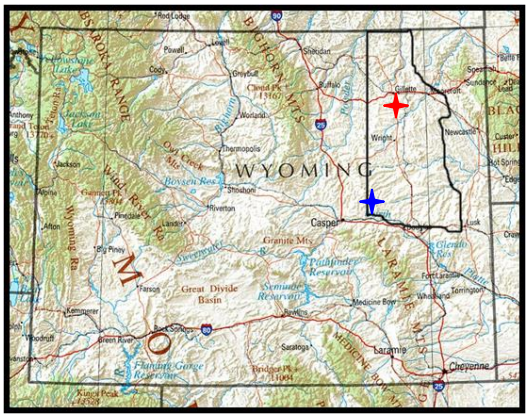

Figure 1. Map of Wyoming with Powder River Basin outlined in black. Belle Ayr Mine, Gillette, WY indicated with a red star and Dave Johnston Mine, Glenrock, WY indicated with a

blue star. Sites within the cool season grass chronosequence are located at the Belle Ayr Mine and the shrub chronosequence at the Dave Johnston Mine.

vegetation (Fig. 2). Average annual precipitation for this area is 266 mm and air temperature 7.4°C. The cool season grass chronosequence of sites included a 1.5 year old stockpile to represent new reclamation, 14 and 26 year old reclamation and an undisturbed site with native vegetation (Fig. 3). Average annual precipitation for this area is 376 mm and air temperature 6.7°C. Each site is approximately 0.5 ha in size. Sites ages at each mine were not replicated as a result of lack of availability of additional reclaimed sites with the same management techniques.

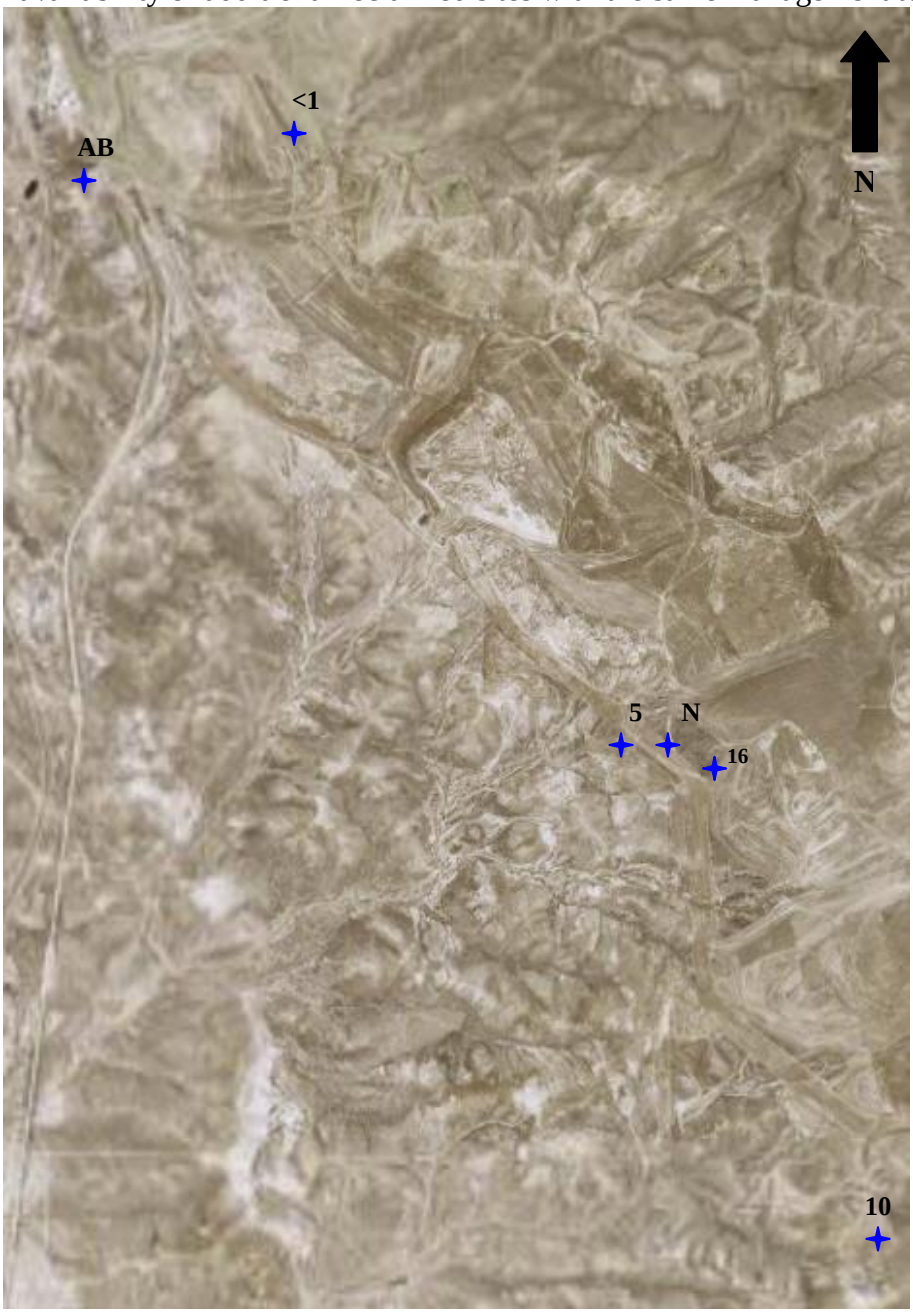

Figure 2. Satellite image from Google Earth (2007) of Dave Johnston Mine, Glenrock, WY (from 18,000 feet). Site locations within the shrub chronosequence are indicated with

blue stars; AB=administrative building, Na = native site; <1 year old site, 5 year old site, 16 year old site, and 10 year old site.

Figure 3. Satellite image from Google Earth (2007) of Belle Ayr Mine, Gillette, WY (from 17,000 feet). Site locations labeled with red stars; AB=administration building, Na=native, 1.5, 14 and 26 year old reclamation.

Soil Sampling and Lab Analysis

Soil samples at each site were collected in May, 2005. The top 5 cm of soil was collected with a trowel at four points along three randomly oriented, 45 meter transects. Five samples were collected for bulk density (BD) at each site within the chronosequences using a double-cylinder, hammer driven core sampler (Grossman and Reinsch, 2002). Samples collected for phospholipids fatty acid (PLFA) analysis were kept frozen until fatty acids could be extracted. Samples collected for soil aggregate and general analysis were air-dried and dry-sieved to 2000 µm to break apart soil clods and leave structure <2000 µm intact. Soil texture was determined on a subset of samples (4 samples covering all transects for each site) using the hydrometer method (Gee and Or, 2002). Electrical conductivity (EC) and pH were determined on all samples with 1:1, soil:water mixtures. An Oakton con 100 series EC probe and an Accument Basic pH meter with a glass electrode were used for analysis. Total C and N values were obtained with an Elementar Variomacro Analyzer. Inorganic carbon (IC) was determined with the modified pressure calcimeter method (Sherrod et al., 2002). Organic C was determined by subtracting IC from total C.

Water stable aggregate size distribution was determined using the wet sieving protocol described by Six et al. (1998). In summary, 100 ± 0.02 g air-dried soil was submerged in deionized water (room temperature) for 5 minutes on a 250 µm sieve. Water stable macroaggregates (250-2000 µm aggregates resistant to slaking) were separated from the bulk soil by moving the sieve 3 cm up and down 50 times in 2 minutes by hand. Material (water plus soil) that went through the sieve was transferred to a 53 µm sieve and the above process was repeated. Material collected from each sieve (250-2000 µm and 53-250 µm) was dried at 55˚C until a constant weight was achieved. Samples were then weighed and stored. Total soil aggregation was calculated by adding the weight of the 250-2000 and 53-250 µm aggregates.

Sand particles are the same size as 250-2000 and 53-250 µm aggregates. Sand corrections were determined for a subset of samples according to Denef et al. (2002). Five mL of sodium hexametaphosphate and 10 mL of water was added to 5 g of the 250-2000 µm aggregates. The same was done for 53-250 µm aggregates. Samples were shaken on a reciprocal shaker for 18 hours and sieved with 250 and 53 µm sieves. Samples collected on each sieve were dried and weighed to determine a sand correction value (weight basis).

A modified Blyer-Digh methodology for PLFA analysis was used to characterize microbial community composition and concentration (Frostegard and Baath, 1991). Because PLFA are readily decomposed upon the death of a microbe, the quantitative analysis of PLFA gives a good estimation of microbial living biomass at the time of sampling. Total microbial biomass was calculated by summing the concentration (area) of all identified bacterial and fungal group peaks. Qualitative and quantitative fatty acid analysis was performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) and Sherlock software (MIDI Inc., Newark, NJ).

Vegetation Sampling and Analysis

Vegetation samples were collected in late June 2005 according to Appendix A of the Wyoming Department of Environmental Quality standards for determination of reclamation success (WYDEQ, 2002). Five aboveground biomass production plots (each 1 m^2 in size) were established using a stratified random method from a baseline (Chambers and Brown, 1983). Vegetation (grasses and forbs) rooted within the plots was clipped within 1 inch of the soil surface and separated by species. Samples collected in the field were dried in a forced air oven at 55˚C for 24 hours, or until a constant weight was reached. Vegetation production data were simplified into functional groups (introduced perennial cool season grasses, native perennial cool season grasses, native perennial warm season grasses, annual introduced grasses, annual forbs and perennial/biennial forbs) for analysis of relationships between vegetation type, soil aggregation and microbial community composition (Table 1).

Statistical Analyses

Statistical analyses were conducted using JMP version 4.0.4 (JMP, 2001). Data were analyzed with one way analysis of variance across site ages to determine differences among reclamation ages. Student's t-tests were used to determine significance between groups following the analysis of variance. Pearson's correlation analysis was used to determine soil aggregate-microbial-vegetation relationships. Bacteria data were log transformed and minimal outliers were excluded where necessary to meet equality of variance and linearity assumptions. All statistical analyses were accomplished at $P<0.05$.

Table 1. Vegetation species included introduced and native cool season grass, warm season grass, annual grass, annual forb, perennial forb and shrub functional groups for correlation analysis.

Grasses/Sedges		**Forbs**	
Introduced Perennial Cool Season	Native Perennial Warm Season	Annual	Perennial/Biennial
Agropyron cristatum	*Aristida purpurea*	*Alyssum desertorum*	*Achillea millefolium*
	Bouteloua gracilis	*Camelina microcarpa*	*Erigeron ochroleucus*
Native Perennial Cool Season	*Calamovilfa longifolia*	*Chenopodium berlandieri*	*Oxytropis sericea*
Pascopyron smithii		*Descurainia sophia*	*Phlox hoodii*
	Annual Introduced Cool Season		*Sphaeralcea coccinea*
Agropyron spicatum		*Lappula occidentalis*	
Agropyron trachycaulum	*Bromus japonicus*	*Logfia arvensis*	*Linum lewisii*
Carex filifolia	*Bromus tectorum*	*Plantago patagonica*	*Medicago sativa*
Elymus lanceolatus		*Salsola iberica*	*Taraxacum officinale*
Festuca idahoensis			*Thermopsis rhombifolia*
Koeleria macrantha			*Tragopogon dumius*
Nasella viridula			*Vicia americana*
Poa secunda			*Astragalus cicer*
Hesperostipa comata			*Melilotus offinalis*

Results

General Trends

Sites within each chronosequence were similar in soil texture (Table 2). The shrub chronosequence soils had a sandy loam texture with low clay percentages, while the cool season grass chronosequence soils were a clay loam texture. Clay percentages were lower in the native cool season site (23%) compared to reclaimed cool season sites (35-43%). Bulk density decreased with reclamation age in the shrub chronosequence but was higher in the native cool season site than in reclaimed cool season sites. Soil organic carbon (SOC) and N generally increased with reclamation age for both chronosequences. Total soil structure (250-2000 µm + 53-250 µm) was variable among sites with a high percentage of total structure being attributed to the 53-250 µm size fraction. Total microbial biomass generally increased with reclamation age and sometimes reached values higher than the native sites. Microbial biomass peaked at 5 years in the shrub soils and 26 years in the cool season grass soils. Electrical conductivity remained below 500 µS cm^{-1} in all sites and generally declined with reclamation age (data not shown). Soil pH was lower in native sites (approximately 6.0) compared to the reclaimed (ranging from 7.0 to 8.0) (data not shown).

Table 2. Soil characteristics (physical, structural, and microbial) for 0-5 cm depths of shrub and cool season grass sites in reclaimed mine land soils in the Powder River Basin, WY. Soil organic carbon (SOC), nitrogen (N), total aggregation (total agg, 250-2000 µm + 53-250 µm aggregates). Letters indicate statistical significance at the P<0.05 level between site ages within each vegetation grouping.

Vegetation Type	Site Age	Clay	Bulk Density	SOC[1]	N	Total Agg	250-2000 µm	53-250 µm	Total Microbial Biomass[2]
	(yrs)	%	g cm^{-3}	(10^6)g ha^{-1}	(10^5) g ha^{-1}	g agg g^{-1} soil	% of structure	% of structure	Area (10^3)
Shrub	<1	17	1.49	3.73	4.13	0.75[a]	21	79	36.8[ab]
	5	14	1.55	5.15	4.37	0.59[bc]	21	79	40.4[a]
	10	13	1.59	5.49	5.83	0.72[a]	24	76	33.2[bc]
	16	12	1.38	8.69	8.51	0.63[b]	27	73	35.8[bc]
	native	13	1.26	7.89	21.9	0.53[c]	28	72	32.1[c]
Cool season grass	1.5	36	1.26	5.94	5.90	0.78[b]	24	76	29.3[a]
	14	43	1.20	6.09	6.85	0.79[b]	44	56	27.8[a]
	26	35	1.21	10.7	7.94	0.82[a]	38	62	32.5[a]
	native	23	1.33	14.7	14.3	0.65[c]	21	79	32.4[a]

[1] Organic carbon values may have been inflated by coal dust contamination of samples

[2] Total microbial biomass was measured by the area under the curve for identified bacteria, fungi and actiomycete groups.

Shrub Chronosequence

Macroaggregate fractions showed no change with time ($F_{4,51}$=0.88; p=0.485). Microaggregate fractions decreased from the newly reclaimed site (<1 year, 0.59 g aggregate g^{-1} soil) to the 5 year old site (0.47 g aggregate g^{-1} soil), and then increased to the 10 (0.54 g aggregate g^{-1} soil) year old reclamation before declining to the native (0.38 g aggregate g^{-1} soil) ($F_{4,53}$ =22.86; p<0.001, Fig. 2a). Bacteria biomass increased from the newly reclaimed site (25.2 x 10^3 area) to the 5 year old reclaimed site (29.6 x 10^3 area) where the values then declined to the 10 year old reclamation (22.7 x 10^3 area) and remained unchanged through the native site (23.2 x 10^3 area)($F_{4,50}$=4.18; p=0.005). This same trend was observed for living fungal biomass ($F_{4,52}$=28.01; p<0.001, Fig. 2b). Actinomycete biomass peaked at the newly reclaimed site, decreased in the 5 year old reclaimed site and then increased in the16 year old reclamation. Actinomycete biomass was lower under native conditions ($F_{4,49}$=7.84; p<0.001). Microbial biomass in reclaimed soils exceeded native community biomass after 5 years since time of reclamation under shrub communities in sandy loam soils.

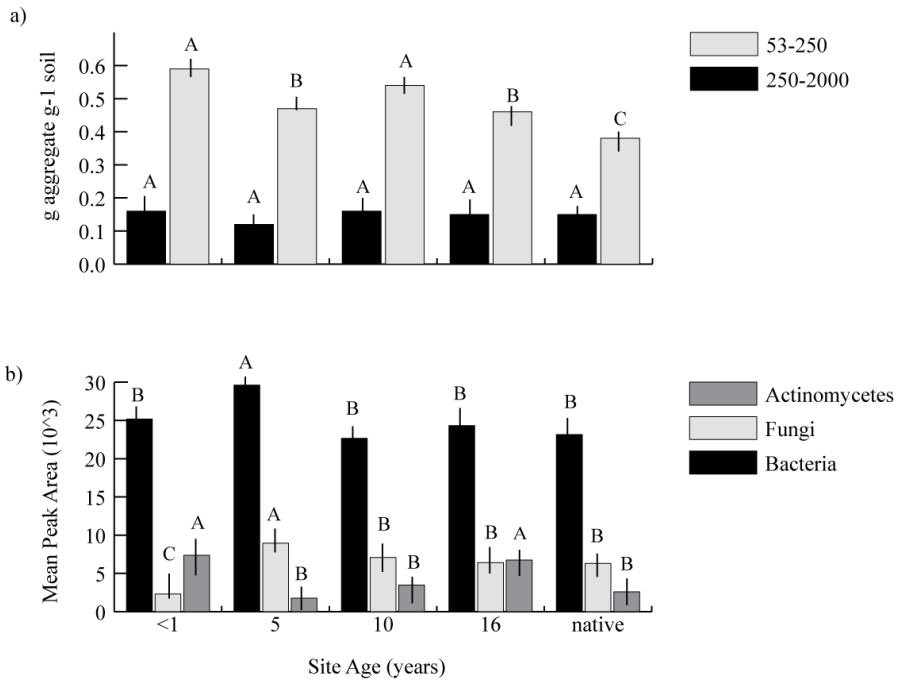

Figure 2. a)Aggregate size distribution and b) microbial biomass for 0-5 cm depth of the shrub chronosequence on Dave Johnston Mine, Glenrock, WY. Letters indicate statistical significance across site ages at the P<0.05 level. Bars indicate standard deviation.

Cool Season Grass

Macroaggregate proportions increased on a weight basis up to the 14 year old reclamation, remained constant through the 26 year old site and thereafter declined ($F_{3,42}$=38.28; p<0.001, Fig. 3a). Microaggregate proportions declined with time and remained constant ($F_{3,42}$=18.07; p<0.001). Bacteria biomass increased from 19.5 x 10^3 area in the 1.5 year old site to 22.0 x 10^3 area in the 14 year old reclamation and then remained unchanged ($F_{3,40}$=3.52; p=0.024, Fig. 3b). Living fungal biomass decreased from the 1.5 to the 14 year old site and then increased to the 26 year old site ($F_{3,40}$=7.88; p<0.001). Actinomycete biomass peaked after 14 years and then declined to level found at the native site ($F_{3,32}$ = 27.78; p<0.001).

Relationships between Aggregation and Microbial Communities

There were no significant relationships between macroaggregates and biological parameters for the shrub chronosequence (Table 3a). Macroaggregation was negatively related to microaggregation and fungi and positively related to bacteria in the cool season grass chronosequence (Table 3b). As bacterial biomass increased, microaggregation decreased for both types of vegetation and soils. Microaggregates were negatively related to living fungi in the shrub chronosequence. Bacteria were positively correlated with fungi in the shrub chronosequence. There was a negative relationship between fungi and actinomycetes for both sites.

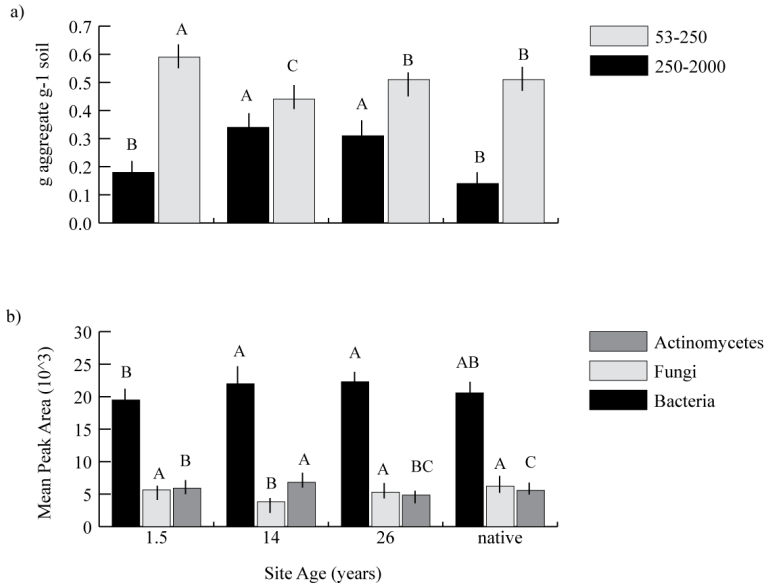

Figure 3. a)Aggregate size distribution and b) microbial biomass for 0-5 cm depth of the cool season grass chronosequence on Belle Ayr Mine, Gillette, WY. Letters indicate statistical significance across site ages at the P<0.05 level. Bars indicate standard deviation.

Table 3. Pearson's product moment correlation coefficients (r) for relationships between soil aggregates, bacteria, fungi, and actinomycetes for the a) shrub chronosequence on Dave Johnston Mine, Glenrock, WY and b) cool season grass chronosequence on the Belle Ayr Mine, Gillette, WY. Statistically significant relationships at the P<0.05 level are shown in bold.

a) Shrub

	Macro	Micro	BAC	FUN	ACT
250-2000 µm aggregates (Macro)	1.00				
53-250 µm aggregates (Micro)	-0.10	1.00			
Bacteria (G+ and G-) (BAC)	-0.17	**-0.26**	1.00		
Fungi (FUN)	-0.16	**-0.39**	**0.52**	1.00	
Actinomycetes (ACT)	0.07	-0.19	-0.06	**-0.49**	1.00

b) Cool Season Grass

250-2000 µm aggregates (Macro)	1.00				
53-250 µm aggregates (Micro)	**-0.71**	1.00			
Bacteria (G+ and G-) (BAC)	**0.40**	**-0.40**	1.00		
Fungi (FUN)	**-0.31**	0.13	-0.12	1.00	
Actinomycetes (ACT)	-0.26	0.17	-0.12	**-0.48**	1.00

Vegetation Relationships

Plant community composition changed across each chronosequence due to vegetation succession. A shift in vegetation was apparent in the shrub chronosequence, where the newly reclaimed community consisted of mostly annual forbs (AF) with no shrub establishment (Table 4). After 5 years of reclamation, shrubs became dense, well established and had a native cool

season grass (NCS, mainly *Festuca*) component. After 10 years, perennial forbs (PF) and warm season grass (WS) established and the shrub density started to decline. The 16 year old site was dominated by NCS grasses and had a lower shrub density. Canopy closure, low shrub density and invasion of *Bromus tectorum* were the main characteristics of the native shrub community (data not shown).

The 1.5 year old site of the cool season grass chronosequence was dominated by NCS grasses (mainly because it was seeded with NCS). Through time as NCS grasses declined, annual grasses (AG) and AF species became increasingly important components of these sites (Table 4), most likely due to an eight year drought during the growing season and spreading of invasive, weedy species. The native vegetation community was a mixture of NCS, WS grasses and PF.

Table 4. Percentage vegetative species per site based on aboveground biomass production for the shrub and cool season grass chronosequences in the Powder River Basin, WY.

	Shrub					**Cool Season**			
	Site Age (yrs)					Site Age (yrs)			
	<1	5	10	16	native	1.5	14	26	native
	Percent					Percent			
Introduced Cool Season Grass	0	0	4	0	0	0	1	0	0
Native Cool Season Grass	14	100	54	79	63	97	50	60	80
Warm Season Grass	0	0	30	0	3	0	0	0	11
Annual Grass	2	0	0	0	30	1	15	6	4
Annual Forb	84	0	1	21	1	2	34	33	0
Perennial Forb	0	0	11	0	0	0	0	1	5

There were no relationships between macroaggregation and plant functional type in the shrub sites. Macroaggregates in cool season grass sites were negatively correlated with NCS and WS while positively correlated with AG and AF (Table 5). Microaggregates were negatively correlated with NCS and positively correlated with AF in the shrub sites, while the opposite relationships were observed at the cool season grass sites. There was a positive relationship between microaggregates and AG in the cool season grass chronosequence. There were no relationships between bacteria and vegetation parameters at the shrub sites. Bacteria biomass was related to NCS, AG and AF in the cool season grass sites. Native cool season grasses declined and were replaced by AG and AF communities, while bacteria biomass increased. There were significant relationships between living fungal hyphae and NCS (positive) and AF (negative) at the shrub sites. Fungi were related to WS and PF vegetation percentages at the cool season grass sites. Actinomycetes were negatively related to NCS and positively related to AF and PF at the shrub sties. No relationships were observed for actinomycete populations and plant functional type for cool season grass sites.

Table 5. Pearson's product moment correlation coefficients (r) for relationships between soil aggregates, bacteria, fungi, actinomycetes, and vegetation properties for the shrub and cool season grass chronosequences in the Powder River Basin, WY. Statistical significance shown in bold at the P<0.05 level, all r values <0.30 were deleted if not significant.

	Shrub						**Cool Season Grass**				
Vegetation Parameter	Macro	Micro	BAC	FUN	ACT		Macro	Micro	BAC	FUN	ACT
% Introduced Cool Season	-	-	-	-	-		-	-	-	-	-
% Native Cool Season	-	**-0.25**	-	**0.66**	**0.47**		**-0.61**	**0.56**	**-0.39**	-	-
% Warm Season	-	-	-	-	-		**-0.19**	-	-	**0.14**	-
% Annual Grass	-	-0.42	-	-	-		**0.69**	**0.71**	**0.37**	-	-
% Annual Forb	-	**0.52**	-	**-0.75**	**0.49**		**0.69**	**-0.40**	**0.41**	-	-
% Perennial Forb	-	-	-	-	**0.10**		-0.43	-	-	**0.27**	-

Discussion

Lack of research on aggregate dynamics and the relationship between soil aggregation and microbial community composition in reclaimed ecosystems has led to a void in the understanding of soil recovery after a massive disturbance such as mining. Minimal research has been conducted on reclaimed soil aggregate dynamics (Malik and Scullion, 1998), although research on reclaimed microbial communities is somewhat better documented (Visser et al., 1983; Frost et al., 2001; Mummey et al., 2002). Most research, in general, relating to soil aggregate and microbial dynamics has been conducted by Jastrow (1987), Jastrow et al. (1998), Miller and Jastrow (1990) and Allison et al. (2005) on a chronosequence (i.e. time since tillage, ranging from 1 to 20 years in age) of recovering tallgrass prairie over silt loam soils in Illinois.

Aggregate formation in the shrub chronosequence was limited by sandy loam soils with low clay content (13-17%). In this type of soil, coarse sand (>250 µm) is cross-linked by fungal hyphae and microaggregates are enmeshed with fine sand (53-250 µm) to form unstable soil macroaggregates (Degens et al., 1996). Macroaggregate strength then depends upon the tensile strength of fungal hyphae rather than the reorientation and binding of clay particles to OM observed in clay loam soils (Degens et al., 1996; Lado et al., 2004; DeGryze et al., 2006; Rillig and Mummey, in press). Determining structural recovery may be dependent upon the relative proportions of microaggregates as they are released from the unstable macroaggregates in sandy loam soils. It is, however, difficult to distinguish between microaggregates released from macroaggregates broken apart during the wet sieving process and residual microaggregates in the soil following disturbance. Aggregate recovery was easier to understand in the clay loam soils underlying the cool season grass chronosequence due to higher clay content (23-36%). Clay contributes to aggregate stability and formation through the reorientation of clay particles around organic matter during dry-wet cycles and the ability of clay to bind with organic matter (Six et al., 2004). Macroaggregate formation peaked after 14 years, following a very similar trend to the bacteria biomass.

Bacterial and fungal biomass in the shrub chronosequence showed a similar peak in biomass after 5 years since reclamation. This same peak may have occurred earlier in the cool season grass soils, but was not captured by the chronosequence as sampled. Jastrow (1996) found an

exponential increase in macroaggregate formation in the tallgrass prairie chronosequence. Restored prairie soils reached an aggregation level of native prairie within 10 years since cultivation. This was attributed to several factors: (1) rapid expansion of fungal hyphae and roots, (2) the species of plants, (3) the presence of clay and polyvalent ions, (4) microaggregate abundance, and (5) a favorable climate. Macroaggregation in the 14 year old reclaimed site exceeded that of the native soil as a result of annual and perennial cool season grass species, higher clay content and an abundant presence of microaggregates. Peak microbial biomass was found 16 years after tillage ceased in a tallgrass prairie chronosequence (Allison et al., 2005); whereas microbial biomass peaked after 5 years in the reclaimed shrub soils and 26 years in the cool season grass soils. Reclaimed microbial communities appeared to recover more rapidly in sandy loam soils under sagebrush compared to clay loam and silt loam soils under short- and tallgrass prairie vegetation.

In both chronosequences, there was a shift in microbial community composition, where a large portion of microbial biomass was dominated by bacteria in recently reclaimed soils eventually becoming more evenly distributed between bacteria, fungi and actinomycetes with time. A similar result was reported by Allison et al. (2005) in restored tallgrass prairie following tillage and Visser et al. (1983) in a post-mining, reclaimed mixed grass prairie. Other reclamation research on microbial communities has found that bacteria biomass in reclaimed soils remained lower than native systems (Visser et al., 1983; Malik and Scullion, 1998), which was not observed in our study. Bacterial and fungal biomass of reclaimed soils often exceeded native soils in our study, much like net primary production of vegetation (Wick et al., 2006). The two variables are likely interacting in reclaimed systems.

The negative relationship between living fungal hypae and macroaggregate development in both chronosequences was unexpected. Total fungal hyphae (living and dead) contribute greatly to macroaggregate formation (Guggenberger et al., 1999; Six et al., 2004; Cosentino et al., 2006; Jastrow, personal communication). Quantification of living and dead fungal hyphae would have been helpful in understanding aggregate dynamics and drivers in reclaimed systems. Currently there is no conversion to change PLFA data, which quantifies living fungal hyphae, into total fungal hyphae. The contribution of living fungal hyphae to microaggregation was inevitable under the concept of aggregate hierarchy discussed earlier (Rillig and Mummey, in press). Hyphae hold together microaggregates in macroaggregates, promoting microaggregate stability. Macroaggregate quantities were influenced by bacteria because the reclaimed communities were dominated by bacteria until fungal and actinomycete populations recovered.

Previous work by Jastrow (1987) found significant, predictable relationships between vegetation type and macroaggregation in restored and native prairie. Aggregates greater than 200 μm correlated well with prairie gramminoids ($R^2 = 0.53$) and aggressive prairie forbs ($R^2 = 0.58$). In this study, plant community composition was related to aggregate size distribution and microbial communities in the cool season grass chronosequence. Relationships were not as strong in the shrub sites; however, as NCS increased and AF decreased, fungi increased. Macro- and microaggregation were strongly related to CSG and AG production in the cool season grass sites. *Bromus tectorum* was included in the AG category and *Agropyron smithii* was a major component of the CSG category. Annual grasses have the ability to dry the soil rapidly in the spring, resulting in rapid wet-dry cycles in top 5 cm of soil (Melgoza et al., 1990**)**, which greatly increases aggregate formation and stability in both macro- and microaggregate size classes (Six et al., 2004). Annual forbs were closely related to macroaggregate formation, a

relationship also expressed by Vogel (1987). Native cool season grasses have very fine root structure, which has little to no effect on macroaggregate formation (Jastrow et al., 1998) and appears to have a negative effect of macroaggregation in reclaimed soils in our study. In this case, time may be the most important influence on aggregate formation (Jastrow, 1987).

Conclusions

Recovery was best indicated by the increase in microbial community biomass in sandy loam shrub soils. Recovery of soil physical and biological properties was evident in both soil microbial and aggregate parameters in clay loam cool season grass soils. Plant community composition, mainly percentage NCS, AG and AG, was highly related to structure and microbial redevelopment as a result of root structure and biomass. More research on the relationship between soil structure and function and plant community composition is necessary for the understanding of reclaimed ecosystems. Future work should include a greater assessment of soil structure and soil C and N in relation to microbial communities along with evaluation of soil recovery under different types of plant communities on the same soil type.

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