COMPREHENSIVE FINAL REPORT

INFLUENCE OF PLANT COMMUNITY STRUCTURE AND TOPSOIL HANDLING METHOD ON SOIL STRUCTURE DEVELOPMENT AND MICROBIAL COMMUNITY RECOVERY IN RECLAIMED SOIL

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Introduction

The ultimate goal of mineland reclamation is reestablishment of a productive, healthy, and sustainable ecosystem suitable for postmining land uses (Munshower, 1993; Harris, Birch and Palmer, 1996). Reestablishment of a stable, healthy, functioning soil is crucial to successful reclamation and long term sustainability of mined lands (Schuman et al., 1985; Whisenant, 1999; Stahl et al., 2002). Soil is the source of nutrition for the large majority of organisms in a terrestrial ecosystem and is also the site of most nutrient cycling processes, e.g. decomposition, mineralization, immobilization, N-fixation, etc (Brady and Weil, 2000; Coleman and Crossley, 1996). Soil organisms, especially microorganisms, drive these nutrient cycling processes and also contribute directly to plant community productivity through numerous interactions with plants including mycorrhiza (Paul and Clark, 1996; Wall-Freckman, 1997). This mutualistic

symbiosis between plants and certain fungi enables plants to obtain greater amounts of nutrients and water from soil and greatly facilitates revegetation (Allen, 1991; Smith and Read, 1997). Soil microorganisms, including mycorrhizal fungi, also contribute to soil function in number of other ways including development and stabilization of soil structure.

Redevelopment and maintenance of good soil structure and stable soil aggregates is crucial to the repair or reclamation of mine land soils (Marrs, 1989; Whisenant, 1999). Macroporosity, which is dependent on good soil structure, is an extremely important factor for a number of important ecological functions of soil. Examples of these functions include water infiltration rates into soil, amount of colonizable habitats for organisms, and movement of gases through soil (Brady and Weil, 2000). Moreover the presence of material rich in carbon (C) in microaggregates (20-250μm), protected from mineralization by microbes, is believed to be an important means by C is sequestered in newly reclaimed mined lands (Stahl et al., 2003). Aggregate formation and turnover results from complex interactions between many factors, including environmental influences, soil properties such as texture, mineralogy, SOM quantity and quality, and the cumulative interactions of the soil biota (Degens, 1997; Baldock, 2002; Six et al., 2002). Arbuscular mycorrhizal fungi are thought to play an extremely important role in soil aggregation through production of extensive hyphal networks and glomalin, a recalcitrant glycoprotein (Rillig, 2004).

Land management practices greatly influences soil structural properties. Mine soils are often compacted from the use of heavy equipment, which greatly reduces aggregate structure and stability (Abdul-Kareem and McCrae, 1984). In agricultural systems, lack of organic matter

inputs to soils decreases aggregate stability (Carter, 2002). Loss of soil aggregation agricultural soils increases the loss of fine material through erosion (Carter, 2002). No till agricultural practices are more conducive to soil structure sustainability than conventional tillage because of crop residue inputs, decreased wet-dry cycles, minimal change to soil conditions (i.e. temperature, moisture, and aeration), and fungal hyphae proliferation (Six et al., 1998). Additionally, no till systems avoid physical destruction of aggregates caused by mechanical disturbance of tillage implements.

Soil Aggregate Formation. Roots have long been known to play a critical role in soil aggregation. This importance is the result of several factors; exudates from roots act as organic binding agents (Jastrow et al, 1998); mycorrhizal associations and the subsequent presence of fungal hyphae (Oades, 1984); the decomposition of plant and fungal residues initiates the development of aggregate hierarchy (Tiessen & Stewart, 1988; Six et al., 2004); all of which leads to greater aggregate stability. Dead root decomposition serves as nuclei for microbial activity, which in turn increases soil aggregation (Six et al., 2004). Wet dry cycles caused by root water uptake increase the binding of clay and root exudates and increases the stability of aggregates (Reid and Gross, 1982). Root exudation and entanglement increase microaggregate and macroaggregate formation, respectively (Six et al., 2004). Fungal hyphae bind together larger soil particles into macroaggregates (Tisdall and Oades, 1982), while bacterial contribute mainly to microaggregate formation (Dorioz et al., 1993). Particulate organic matter (POM) protected by microaggregates is more microbially altered and complex compared to POM held within macroaggregates (Jastrow and Miller, 1997).

Soil Microorganisms. Microbes in soil, mainly fungi and bacteria, are the organisms primarily responsible for decomposition of dead plant, animal and microbial biomass and mineralization of organic nutrients to an inorganic form (Paul and Clark, 1996). These organisms are very sensitive to environmental conditions and variably distributed in the soil matrix based on their environmental requirements and soil structure characteristics (Mummey and Stahl, 2004; Sylvia et al., 2005; Mummey et al., 2006). Data we have collected on reclaimed surface mine sites in previous studies have shown that microbial community structure in reclaimed soils is quite different that in nearby undisturbed soils (Mummey, Stahl and Buyer, 2002; Stahl et al., 2003; Anderson, Stahl and Ingram, 2004;). Our previous studies on reclaimed soils have, as have almost all other studies of soil microbial communities, been undertaken on bulk soil samples. However, microbial communities and the functions they perform most typically occur in spatially segregated microenvironments in the soil leading to a microscale biogeography (Mummey and Stahl, 2004; Mummey et al., 2006), which analysis of bulk samples largely fail to detect. As a result, little is known about relationships between microbial distribution within soil, organic matter dynamics and soil structure. Data available on these relationships indicate that they are very important to organic matter decomposition and protection. Understanding these relationships is vital to an in depth understanding and sustainable management of soil processes involved in disturbed soil development.

Objectives. The overall objective of this research has been to determine the influence of topsoil handling practice and plant community type on soil structure development and microbial community recovery (including recovery of mycorrhizal fungi) on surface mine reclamation sites. Specifically, using established research sites and chronosequences of surface coal mine

reclamation sites that were reclaimed with either stockpiled topsoil or directly placed (hauled) and are now revegetated with cool season grasses or sagebrush and cool season grasses, we:

1) Examined microbial community recovery (including that of arbuscular mycorrhizal fungi) and soil structure development in soils on a number of different aged sites (a chronosequence) reclaimed using stockpiled topsoil and compared it to that of similarly aged sites with the same vegetation reclaimed using soil directly placed without ever having been stockpiled.

2) Analyzed microbial community recovery and soil structure development in soils on a chronosequence of different aged sites reclaimed using the same topsoil management strategy but revegetated with either cool season grasses or sagebrush and cool season grasses .

Methods

Our general approach to this research has been to examine and compare soil structural properties and soil microbial communities at sites reclaimed using either directly placed topsoil or stockpiled topsoil and revegetated with either cool season grasses or sagebrush and cool season grasses. We will include reclaimed sites that have had different amounts of time to recover from disturbance associated with surface mining. We used established chronosequences of reclaimed

sites on two mines (Belle Ayr Coal Mine and Dave Johnson Coal Mine) in northeastern Wyoming. Examination of soils that have been recovering from disturbance for different lengths of time will enable us to track the process of soil structure redevelopment and microbial community recovery through time and compare how it differs at sites reclaimed with different plant communities and using different topsoil handling strategies. A greenhouse study was conducted to closely examine the influence of different plant species on soil aggregation and microbial community structure.

Research sites for soil sampling were selected that have minimal macro- and mesovariation in factors such as slope, aspect, and management history to assure the validity of our comparisons. Soil was collected from two depths at each research site, 0-5 cm and 5-15 cm. The top 5 cm of soil was collected with a trowel and the 5-15 cm depth with a 2.5 cm diameter step probe at four points along three randomly oriented, 45 meter transects. Five samples for the three depths were collected for bulk density (BD) at each site using a double-cylinder, hammer driven core sampler (Grossman and Reinsch, 2002).

In the lab, samples were air-dried and dry-sieved to 8000 μm to break apart soil clods and retain structure <8000 μm. Soil texture was determined on a subset of samples using the hydrometer method (Gee and Or, 2002). Bulk C and N values were obtained by dry combustion with an Elementar Variomacro Analyzer (Hanau, Germany). Inorganic carbon (IC) was determined with the modified pressure calcimeter method (Sherrod et al., 2002).

For analysis of soil structure, water stable aggregate size distribution were determined using wet sieving protocol described by Six et al. (1998). To summarize, 100 ± 0.02 grams of air-dried soil was submerged in deionized water (room temperature) for 5 minutes on a 2000 µm sieve. Water stable large macroaggregates were separated from the bulk soil by moving the sieve 3 cm up and down 50 times in 2 minutes. Material (water plus soil) passing through the sieve was transferred to a 250 µm sieve and the above process repeated. Material collected from each sieve (250-2000 µm, 53-250 µm and <53 µm) was dried at 55˚C until a constant weight is achieved. Samples are then weighed and stored. Mean weight diameter (MWD) was calculated for each site based on Kemper and Rosenau (1986).

Particulate organic matter (POM) analysis was conducted according to Six et al. (1998). Eight gram samples of large macroaggregates, small macroaggregates and free microaggregates were oven dried overnight at 105˚C. The samples were then suspended in 35 mL of 1.85 g cm^{-3} density sodium polytungstate (SPT) in a 50 mL centrifuge tube and shaken gently by hand to bring sample into suspension (approximately 10 strokes). Material on the lid was washed into the cylinder using 10 mL of SPT. Samples were then placed under vacuum (138 kPa) for approximately 10 minutes to remove the air trapped within the aggregates. Samples were centrifuged at 20˚C for 60 minutes at 2500 rpm. Floating material (considered LF) was aspirated through a 20 µm nylon filter and rinsed with deionized water. Material from the filter was transferred into a beaker and dried at 55˚C overnight. Remaining soil in the centrifuge tube (iPOM, sand, silt and clay) was rinsed twice with deionized water, flocculated with 0.25 M $CaCl_2$ and 0.25 M $MgCl_2$ and centrifuged at 20˚C for 15 minutes at 2500 rpm. Twelve 6 mm glass beads were added to each centrifuge tube, which were placed on a reciprocal shaker for 18 hours

on a low setting. Samples were removed and sieved with nested 250 and 53 μm sieves for large and small macroaggregate samples and a 53 μm sieve for microaggregate samples. Material remaining on the sieve (iPOM and sand) and material washed through the sieve (silt and clay) were dried at 55˚C overnight. Samples were weighed and stored in scintillation vials until analyzed for C and N.

Samples (250-2000 μm and 53-250 μm aggregates) were analyzed for total C and N using dry combustion on an Elementar Variomacro Analyzer (Hanau, Germany). Density fractionation samples (LF, iPOM, and silt+clay) were also analyzed for C and N. Comparisons of C and N content across sites are not valid unless they are sand corrected (Elliott et al., 1991). The following formulas were used to calculate the sand free C content (Equation 1) and sand free N content (Equation 2) for each size class (Denef et al., 2001):

(1) $\text{Sand-free } C_{fraction} = C_{fraction} * [\text{g aggregate}_{fraction} / (\text{g POM aggregate}_{fraction} - \text{g sand+iPOM}_{fraction})]$

(2) $\text{Sand free } N_{fraction} = N_{fraction} * [\text{g aggregate}_{fraction} / (\text{g POM aggregate}_{fraction} - \text{g sand+iPOM}_{fraction})]$

Microbial community analysis were conducted using examination of microbial phospholipid ester-linked fatty acids (PLFAs) from soil (Cavigelli, Robertson, and Klug, 1995; Buyer and Drinkwater, 1997; Mummey, Stahl and Buyer, 2002a; 2002b). PLFA profiles were used to evaluate soil microbial community composition for all soils examined in this study. Total lipids were extracted from moist soil by a chloroform–methanol extraction (Bligh and Dyer, 1959). PLFAs were purified, quantified and identified using the MIDI Sherlock Microbial Identification

System. Total extractable PLFAs from a soil sample were used as a measure of microbial biomass at each site. Soil microbial communities in soils examined were characterized based on types and amounts of PLFAs present. Multivariate statistical techniques were used to analyze the PLFA data.

Recovery of arbuscular mycorrhizal fungi was assessed by quantification of a PLFA biomarker for arbuscular mycorrhizal fungi (16:1w5c, Olsson et al., 1995; Olsson, 1999).

Stable carbon isotope analysis was conducted on (1) macroaggregate and microaggregate size fractions, (2) density fractionation samples (light fraction, POM, and silt+clay) for each aggregate size, (3) a subset of bulk soil samples, and (4) plant root samples collected from each site. Soil samples were ground and pretreated for carbonates with 1 M HCl overnight. Samples were then rinsed with deionized water until pH reached pre-acid addition levels. Approximately 40-50 µg of pre-treated soil was analyzed using a Finnigan Delta+XP continuous flow inlet Isotope Ratio Mass Spectometer (IRMS) in the University of Wyoming Stable Isotope Facility. Samples were presented as $\delta^{13}C$ values relative to the V-PDB standard as:

$$\delta^{13}C = [(R_{sample}-R_{standard})-1] \times 1000 \quad \text{(with R being the } {}^{13}C:{}^{12}C \text{ ratio)}$$

To examine the influence grasses and shrubs on soil structure and microbial community development with minimal environmental variation, we conducted a greenhouse experiment in which four species of grass or shrub were grown in pots of a minesoil we had sieved to destroy all structure. We then monitored development of aggregate structure and microbial communities

in that soil for 9 months. Four plant species either commonly used in reclamation seed mixes or are considered to be weed species of reclaimed areas will be included in the experiment:

Reclamation species

Cool-season grass: Western wheatgrass - *Pascopyrum smithii* (Rydb.) A. Löve

Warm-season grass – *Blue grama* (Bouteloua gracilis)

Shrub – Wyoming big sagebrush - *Tridentata wyomingensis* Nutt. ssp. *wyomingensis*

Weed species

Cheatgrass – *Bromus tectorum* L.

We collected a disturbed loamy soil from a topsoil stockpile at a closed surface coal mine (Rosebud Coal Mine – located near Hanna, Wyoming) for this experiment. Using the same soil for all four plant species allowed us to compare the simple influence of plant variety on soil aggregation. Pots ca. 30 cm diameter and 30 cm deep were used and planted with a high density of seeds (ca. 30) that were later thinned to 3 or four plants per pot. This produced a sufficiently high root density in each pot. Plants were grown at the University of Wyoming College of Agriculture Greenhouse Facility under conditions which mimic the environmental conditions present in the Powder River Basin (day temperature, 30˚ C, night temperature, 10˚ C). Soil in the pots was sampled at the initiation of the experiment (T0) as well at one (T1), three (T3), and six months (T6), after seedling emergence. Pots were sampled by removing four 1" cores. At each sampling time and for each plant species, five replicate pots were sampled and analyzed for microbial community characterization (as described previously and soil aggregate size classes

(including the 2000-8000 μm size class, as described previously). In addition, at T0 and T6, soil particulate organic matter (POM)(as described previously) and organic carbon and nitrogen were also be analysed. A total of 65 pots (4 plant spp. x 3 sampling periods x 5 replicates) were sampled over the course of the experiment.

RESULTS

General Soil Characteristics

Clay percentages were slightly lower in the stockpiled soils compared to the directly hauled soils used for reclamation, but were more comparable to the native site soils. This could have resulted in lower aggregation and C accumulation in the stockpiled soils. Bulk density remained similar throughout sites and topsoil handling method. Bulk soil C increased through site age for both the stockpiled and directly hauled soils. Electrical conductivity and pH values were similar between all sites.

Aggregate Size Distribution

Directly Hauled: Soil macroaggregate (250-2000 μm) proportions were higher in the reclaimed compared to the native soils (Figure 1). There was a significant increase in macroaggregate proportions between the <1 and 14 year old sites for directly hauled soils. Lower clay contents in the native soils may have contributed to abnormally low macroaggregation compared to reclaimed soils.

Table 1. General properties of soil from the directly hauled and stockpiled C_3 grass chronosequences, Belle Ayr Mine, Gillette, WY. Soil organic carbon (SOC), nitrogen (N), electrical conductivity (EC).

Site Age	Topsoil Handling Method	Depth	Sand	Silt	Clay	Bulk Density	SOC†	N	C:N	EC	pH
(yrs)		(cm)		%		g cm^{-3}	Mg ha^{-1}	Mg ha^{-1}		S m^{-1}	
<1	direct/stockpile	0-5	39	34	27	1.26	5.59	0.467	12:1	0.05	7.8
		5-15	36	35	29	1.35	9.81	1.06	9:1	0.06	7.7
9	stockpile	0-5	44	36	20	1.31	8.66	0.698	12:1	0.03	7.9
		5-15	51	29	20	1.55	18.02	1.202	15.0	0.03	8.2
14	direct	0-5	38	28	34	1.20	19.7	1.09	18:1	0.04	7.6
		5-15	38	26	36	1.33	14.3	1.16	12:1	0.04	8.1
15	stockpile	0-5	41	37	22	1.19	14.3	0.897	15:1	0.04	7.5
		5-15	46	31	23	1.40	17.09	1.19	14.3	0.03	8.0
26	direct	0-5	38	33	29	1.21	13.4	0.909	15:1	0.04	7.0
		5-15	37	30	33	1.38	13.5	1.17	12:1	0.03	7.8
native	native	0-5	53	26	21	1.34	9.92	0.958	10:1	0.02	6.6
		5-15	54	21	26	1.44	14.3	1.34	11:1	0.02	7.0

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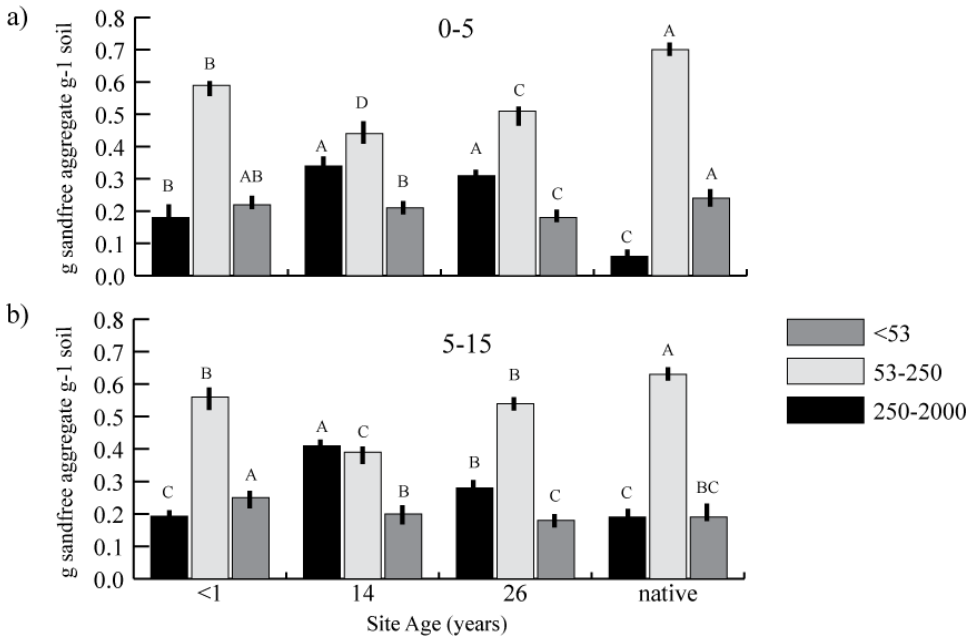

Figure 1. Aggregate size distribution for soils in the directly hauled chronosequence at Belle Ayr Mine, Gillette, WY. Significance shown across site ages for a) macroaggregates (250-2000 μm), microaggregates (53-250 μm) and silt and clay (<53 μm) at the $P \leq 0.05$ level. Bars represent standard deviation.

Stockpiled: No apparent trends in macroaggregate proportions were observed with time for stockpiled soils (Figure 2). Reclaimed stockpiled soils were still relatively low in aggregation compared to recently disturbed soils as well as directly hauled soils.

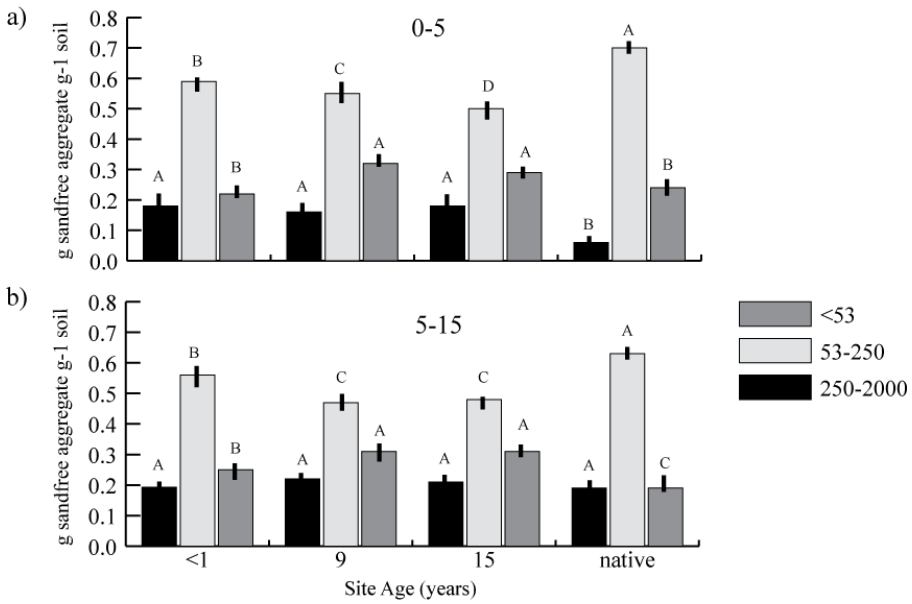

Figure 2. Aggregate size distribution for soils in the stockpiled chronosequence at Belle Ayr Mine, Gillette, WY. Significance shown across site ages for a) macroaggregates (250-2000 μm), microaggregates (53-250 μm) and silt and clay (<53 μm) at the $P \leq 0.05$ level. Bars represent standard deviation.

Comparison: Aggregation was much lower in stockpiled soils compared to directly hauled soils. This is likely the result of lower clay contents in the stockpiled soils rather than topsoil handling method. There could have also been differences in vegetation communities and root types between sites, which would result in differences in aggregation. Additionally, the directly hauled soils were sampled when the soils were fairly wet and the stockpiled soils when the soils were very dry. This could have led to increased aggregate breakup during sampling of the stockpiled soils.

Aggregate Carbon

Both aggregate size classes and aggregate fractions C concentrations were significantly reduced by the mining process using the <1 and native sites as endpoints. Macroaggregate C

concentrations decreased by 43% while microaggregate C decreased 59% (Figure 3a). The greatest percentage loss of C and N were in the LF for both aggregate size classes. Macroaggregate LF C decreased by 92% following soil removal processes. Microaggregate LF C was reduced by 76% (Figure 3b). Macroaggregate HF C increased by 38% while microaggregate HF C was reduced by 59% (Figure 3c). Less change was observed in the macroaggregate Mineral C concentrations (12% increase). A substantial loss was still observed in the microaggregate Mineral C 49% (Figure 3d).

Directly Hauled: Some interesting trends in C concentrations were observed following a mining disturbance. Aggregate associated C increased significantly from the <1 year old reclaimed soil to the 26 year old reclaimed site for the macro- and microaggregate size fractions, to a higher concentration than that observed in the native site soil. Reclaimed soil macroaggregate C increased by 4.27 g C kg^{-1} sand free aggregate yr^{-1}, while microaggregate C increased by 4.52 g C kg^{-1} sand free aggregate yr^{-1}. Microaggregate C was higher than macroaggregate C, which is not commonly observed. Macroaggregate LF C concentrations increased through time, but did not reach concentrations observed in the native site soil. Microaggregate LF C increased with each reclaimed site age and was higher than observed concentrations in the native site soil. A majority of aggregate C concentrations were observed in the HF material. Macro- and microaggregate HF C followed a similar trend to aggregate C concentration shifts through time. Macro- and microaggregate Mineral C concentrations were the same as that observed in the native site soil in the 14 and 26 year old sites.

Stockpiled: Similar trends were observed in stockpiled soils as those observed in directly hauled soils. Reclaimed soil aggregate C reached concentrations higher than the native soil aggregate C. Reclaimed soil macroaggregate C increased by 5.27 g C kg^{-1} sand free aggregate yr^{-}

^{1}, while microaggregate C increased by 9.49 g C kg^{-1} sand free aggregate yr^{-1}. Macroaggregate LF C concentrations increased significantly with reclamation age towards native LF C concentrations (1.73 g LF C kg^{-1} sand free aggregate yr^{-1}), where microaggregate LF C concentrations succeeded native LF C concentrations after 15 years of reclamation. Again, a majority of aggregate C was observed in the HF and followed similar trends to aggregate C. Mineral C concentrations exceeded native concentrations for macroaggregates and reached those of native soils for microaggregates within 15 years.

Comparison: Concentrations were lower for macroaggregate associated C and higher for microaggregate C after 15 years for directly hauled soils compared to stockpiled soils used in reclamation. Rates of aggregate C accumulation in stockpiled soils were also higher compared to directly hauled soils used in reclamation. Both directly hauled and stockpiled soils used in reclamation appear to be recovering towards native conditions.

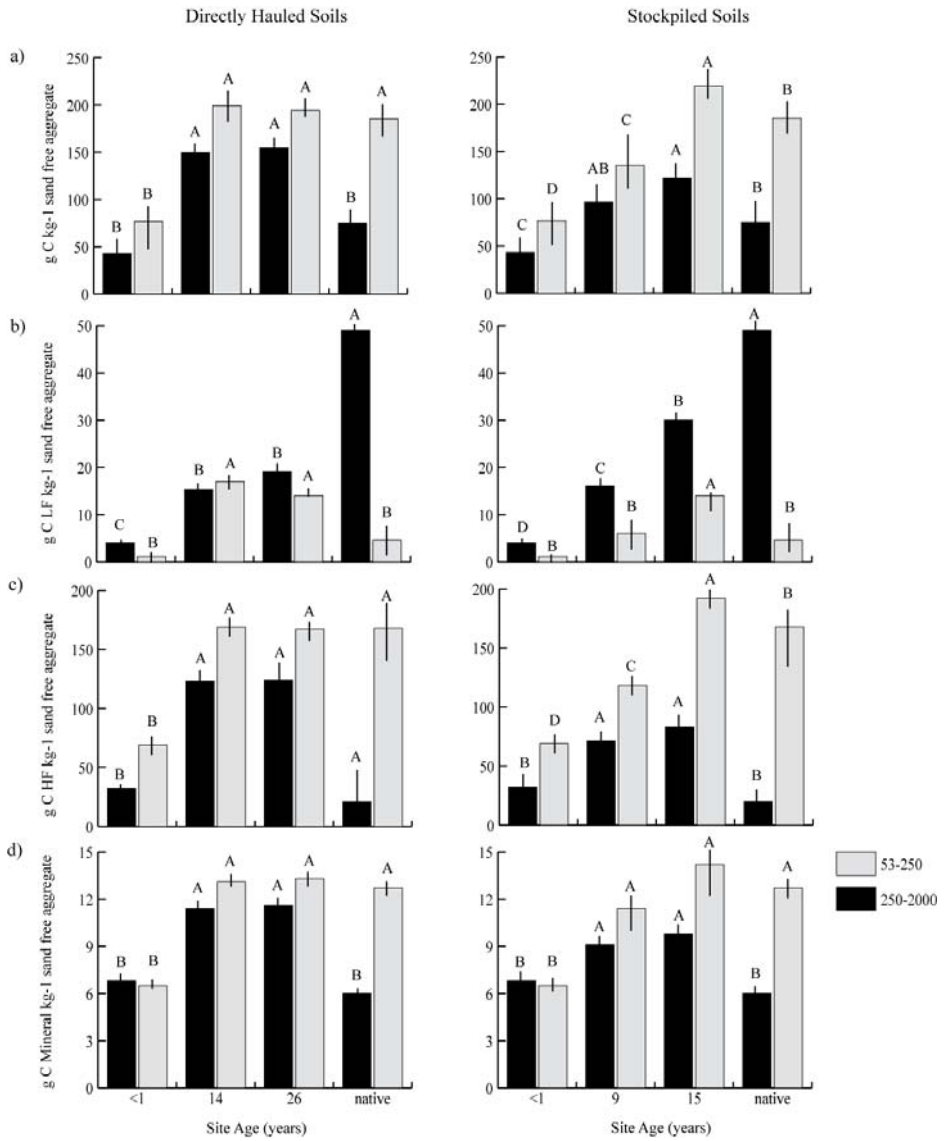

Figure 3. Carbon concentrations for directly hauled and stockpiled soil chronosequences; a) macro- and microaggregate associated b) aggregate associated light fraction (LF), c) aggregate associated heavy fraction (HF), and d) aggregate associated mineral fraction (Mineral). Significance shown among site ages at $P \leq 0.05$. Bars represent standard deviation.

Aggregate Organic Matter Dynamics

Directly Hauled: Stable ^{13}C isotope signatures of whole soil, macro- and microaggregate OM were significantly lower in $\delta^{13}C$ with reclamation age (Figure 4a). The weighted average $\delta^{13}C$ of the native whole soil was -20.5‰ and became slightly more depleted in ^{13}C in the <1 year old site (-21.0 ‰). Whole soil became significantly more depleted in ^{13}C with reclamation age,

reaching a value of -24.2‰ after 26 years of C_3 plant inputs. Macroaggregate OM had an average weighted signature of -21.7‰ in the native soil, while microaggregate OM was less depleted with a signature of -19.6‰. Macro- and microaggregate associated OM became significantly more depleted between the <1 (-22.0 and -21.5‰, respectively) and 14 year old reclamation (-23.9 and -24.0‰, respectively). These values indicated rapid incorporation of new C_3 plant material into protected soil C pools. Macroaggregate associated C continued to significantly decline in signature to the 26 year old reclaimed site (-25.0‰), while microaggregate C remained unchanged (-24.5‰).

New C from C_3 plant material contributed 59% of total C in the whole soil during the 26 years following reclamation, with 42% of new C contributions after 14 years since reclamation. Incorporation of new C was even more drastic in the aggregate size fractions. Macroaggregate associated new C was 44% of total organic C within 14 years and increased to 66% after 26 years. Microaggregate new C increased from 62% of total organic C between the <1 year old site and 14 year old reclamation to 65% after 26 years. It is important to consider that coal dust ($\delta^{13}C$ -27 to -30 ‰) contamination may have artificially altered the signature of the reclaimed soil, leading to an inflated estimate of C incorporation into the whole soil and soil aggregates.

Stockpiled: Whole soil became significantly more depleted in ^{13}C with reclamation age; $\delta^{13}C$ -20.5 to -22.5 ‰ over 15 years (Figure 4b). Macroaggregate $\delta^{13}C$ signatures decreased from -21.7 to -24.1 ‰ and microaggregate signatures decreased from -19.6 to -22.3 ‰ within 15 years. Both decreases in isotopic signatures indicate rapid incorporation of new C into aggregate structure.

New C contributed to 33% of total C in whole soils after 15 years since reclamation. Aggregate fractions showed greater proportions of new C; macroaggregates 48% and

microaggregates 38%. New C from highly productive reclaimed plant communities are contributing OM to physically protected pools.

Comparison: After 14-15 years of reclamation, directly hauled soils have higher percentages of new C compared to stockpiled soils. This may be the result of differences in microbial and plant communities. Additionally, directly hauled soils had greater aggregate stability for physical protection of soil C compared to stockpiled soils used in reclamation. This would allow the greater new C component of the directly hauled soils because of OM protection.

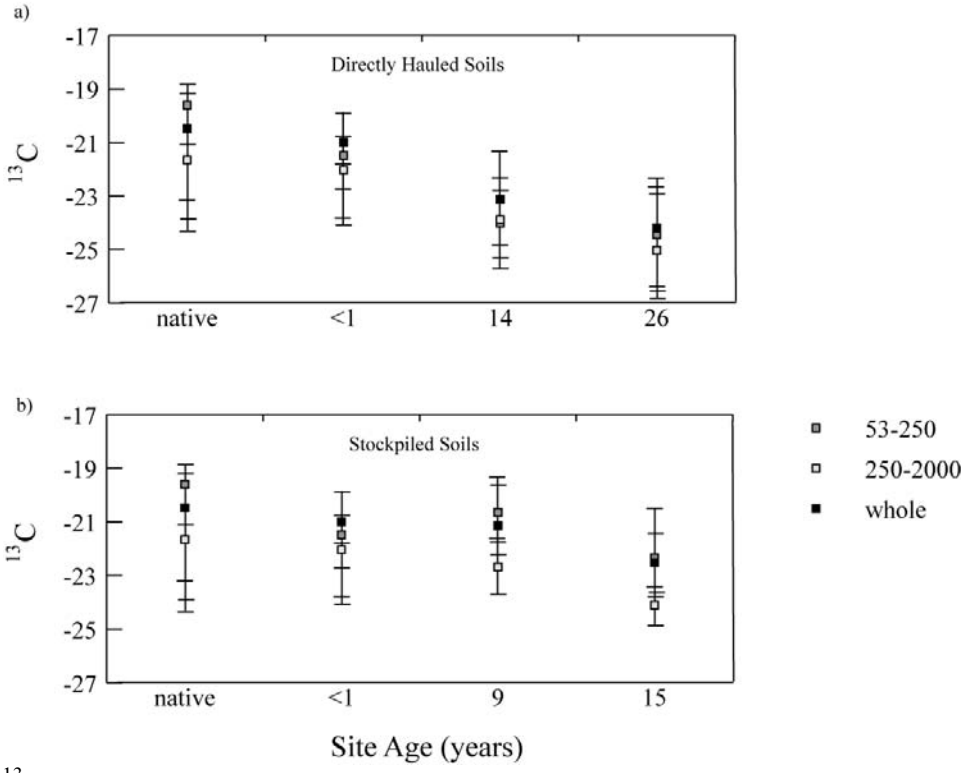

Figure 4. $\delta^{13}C$ signatures of whole and aggregate associated organic matter for directly hauled and stockpiled soils in two C_3 grass chronosequences at Belle Ayr Mine, Gillette, WY. Macroaggregates (250-2000 μm), microaggregates (53-250 μm) and whole (total soil).

Aggregate Fraction Organic Matter Dynamics

Directly Hauled: Macro- and microaggregate associated LF $\delta^{13}C$ values decreased between 14 (-23.5 and -23.0‰, respectively) and 26 year old reclamation (-23.8 and -23.6‰, respectively, Figure 5a). Macro- and microaggregate HF $\delta^{13}C$ values significantly decreased between <1

reclaimed soil (-21.1 and -22.5‰, respectively) to the 26 year old reclaimed soil (-23.3 and -23.8‰, respectively, Figure 5b). Mineral fraction $\delta^{13}C$ signatures became more depleted with reclamation age for both macro- and microaggregates (Figure 5c).

New C incorporated into LF C accounted for 34% of total C for macroaggregates and 42% for microaggregates after 26 years. After 14 years, new LF C was negligible for macroaggregates and 10% for microaggregates. Protected HF C was 58% new C for macroaggregates and 61% for microaggregates after 26 years and 16 and 36%, respectively after 14 years. Mineral associated C was 57% new C for macroaggregates and 53% for microaggregates after 26 years and 35 and 43%, respectively, after 14 years.

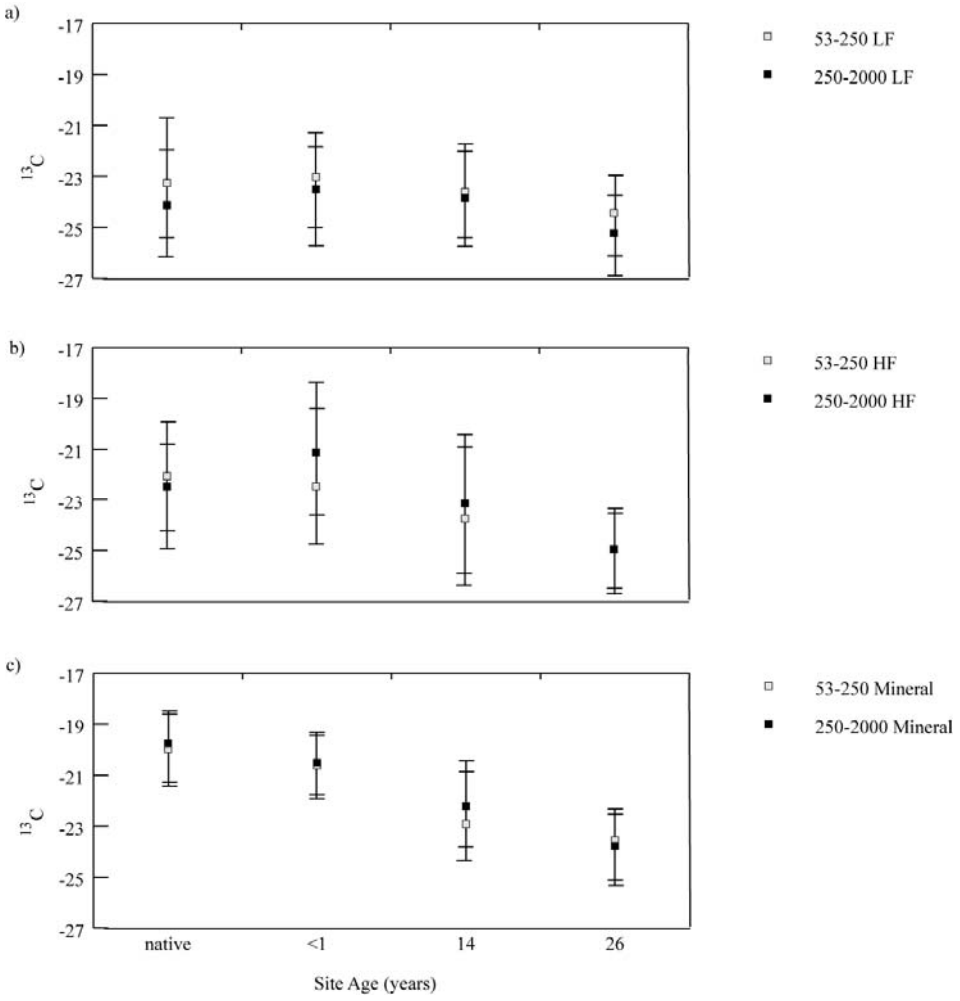

Figure 5. $\delta^{13}C$ signatures of directly hauled soils for aggregate associated a) light fraction, b) intra-aggregate particulate organic matter, and c) mineral fraction for a C_3 grass chronosequence at Belle Ayr Mine, Gillette, WY. Macroaggregates (250-2000 μm), microaggregates (53-250 μm), light fraction (LF), intra-aggregate particulate organic matter and sand (HF).

Stockpiled: Macroaggregate LF $\delta^{13}C$ values decreased from -24.1 to -26.2 ‰ and microaggregate $\delta^{13}C$ values decreased from -23.3 to -24.6 ‰ after 15 years of new plant inputs (Figure 6a). Macro- and microaggregate HF $\delta^{13}C$ values showed an increasing trend after 9 years and then decreased to -23.8 and -23.9 ‰, respectively after 15 years (Figure 6b). Macro- and microaggregate Mineral fraction $\delta^{13}C$ values remained unchanged through reclamation age (Figure 6c).

A majority of the new C was incorporated into the LF material for both macro- and microaggregates (78 and 39%, respectively). Between 32 and 40% of new C was incorporated into macro- and microaggregate HF material, while even less (14 and 6%) was found in Mineral fraction material. The lack of aggregate structure in the stockpiled soils used for reclamation may have resulted in contribution of new C to unprotected pools (such as the LF) rather than into physically protected HF and chemically protected Mineral pools.

Comparisons: More new C is being incorporated into physically and chemically protected pools in the directly hauled soils rather than into available pools as observed in the stockpiled soils. This is likely the result of low aggregation in the stockpiled soils and comparatively high aggregation in the directly hauled soils. Additionally, there may be differences in microbial community dynamics that allow in accumulation of new C in OM pools available for microbial decomposition.

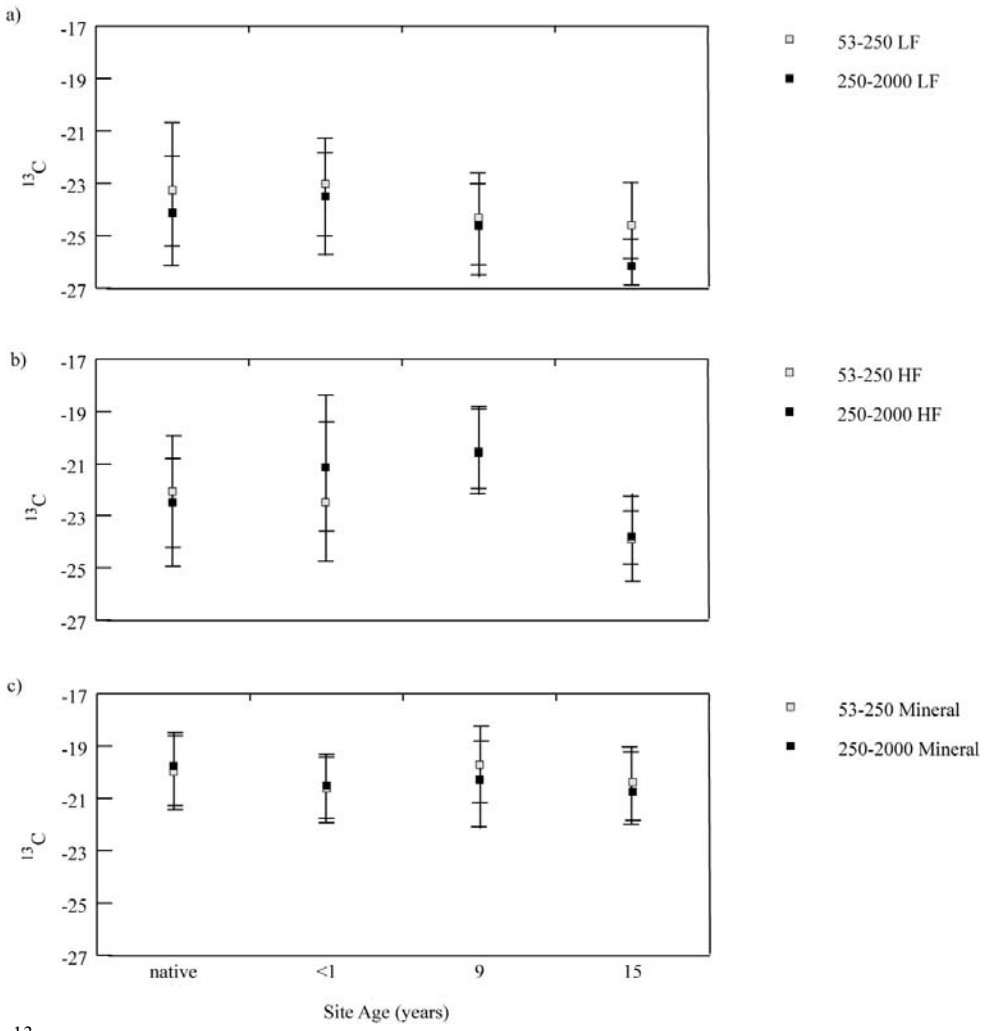

Figure 6. $\delta^{13}C$ signatures of stockpiled soils for aggregate associated a) light fraction, b) intra-aggregate particulate organic matter, and c) mineral fraction for a C_3 grass chronosequence at Belle Ayr Mine, Gillette, WY. Macroaggregates (250-2000 μm), microaggregates (53-250 μm), light fraction (LF), intra-aggregate particulate organic matter and sand (HF).

Microbial community structure

Stockpiled: There was a general increase in all microbial groups in soil that had been stored for a period of time (Fig. 6) before being respread and reseeded. There were, however, two exceptions to this general phenomena, Actinomycetes and 'total' microbial biomass (at this point in time we would like to point out that the measurement of 'total' microbial biomass can not be simply measured by summing all the different PLFA groups together as some microbial groups will have

a number of the same PLFA biomarkers and thus this give rise to an overestimation of microbial biomass). In both cases, there was a very rapid increase with five years for both actinomycetes and 'total' biomass relative to the newly reseeded site. As actinomycetes are bacteria, they, along with gram positive and negative bacteria, are generally less impacted by disturbance than other microbial groupings, and can be considered to r-strategists. The rapid increase in 'total' biomass simply reflects the large increase in all of the three major bacterial groups (i.e., gram positive, gram negative, and actinomycetes). The decline in 'total' biomass from a peak of five years after reclamation to less than half in the native site is probably explained by a number of reasons. Immediately after a disturbance there is a large increase in labile sources of carbon and nutrient which will favor the rapid growth and reproduction of bacteria (over fungi). Secondly, new plants inputs can be fairly labile and this too will favour bacterial growth over fungal growth. Over time as plant litter becomes humified, there is a reduction of labile sources of nutrients, leading to a general decrease in bacteria. In contrast, however, fungi, being critical in the decomposition process, are able to take advantage of older, more humified material. The observed increase in AMF represents the increase in mycorrhizal associations between AMF and perennial cool-season grass species.

As was expected there is large drop off in the content of all microbial groups between the 0-5 and 5-15 cm. This is simply a reflection of the more wetting-up events that occur in this top layer. Increased litter inputs (above- and belowground) and the exudates secreted by roots, that are present in large amounts near the soil surface, provide an important source of carbon and nutrients required for microbial growth and reproduction.

Direct Hauled: The same general pattern observed in the stockpiled soils was also generally observed in the directly hauled soils. Again, there was a large increase in all microbial groups after the initial sampling at 1.5 yrs that peaked at around 26 yrs. This was followed by a general decline in most groups to lower (though usually not significantly) amounts compared to native soils.

Comparisons between direct haul and stockpiled soils: Previous work by other researchers has noted that while in the short term (less than five years) direct hauled soils are, generally, are biologically more 'healthy'. This is due to the fact that because of the reduced amount of handling, directly hauled soils retain greater amounts of carbon, nutrients, and this helps to maintain a greater amount of the microbial communities present in the soil prior to disturbance.

However, after soils have been successfully reclaimed for periods greater than five to ten years, there is often little difference between the reclamation process as this time allows microbial communities to recover to a comparable level prior to being disturbed. This was noticeable in this study where comparing the 14 yr old site of the stockpiled chronosequence (Fig. 6) with the 15 yr old directly hauled soil (Fig. 7) and it is apparent that there are remarkably few differences in the amounts of PLFA's between the two treatments. That there was no general linear increase in the directly hauled (as there was in the stockpiled soils), but rather a large increase after 14 yrs, is simply explained by the fact that after the 1.5 yr old site the next youngest was 14 yrs old and by this time it appears that the microbial communities had recovered.

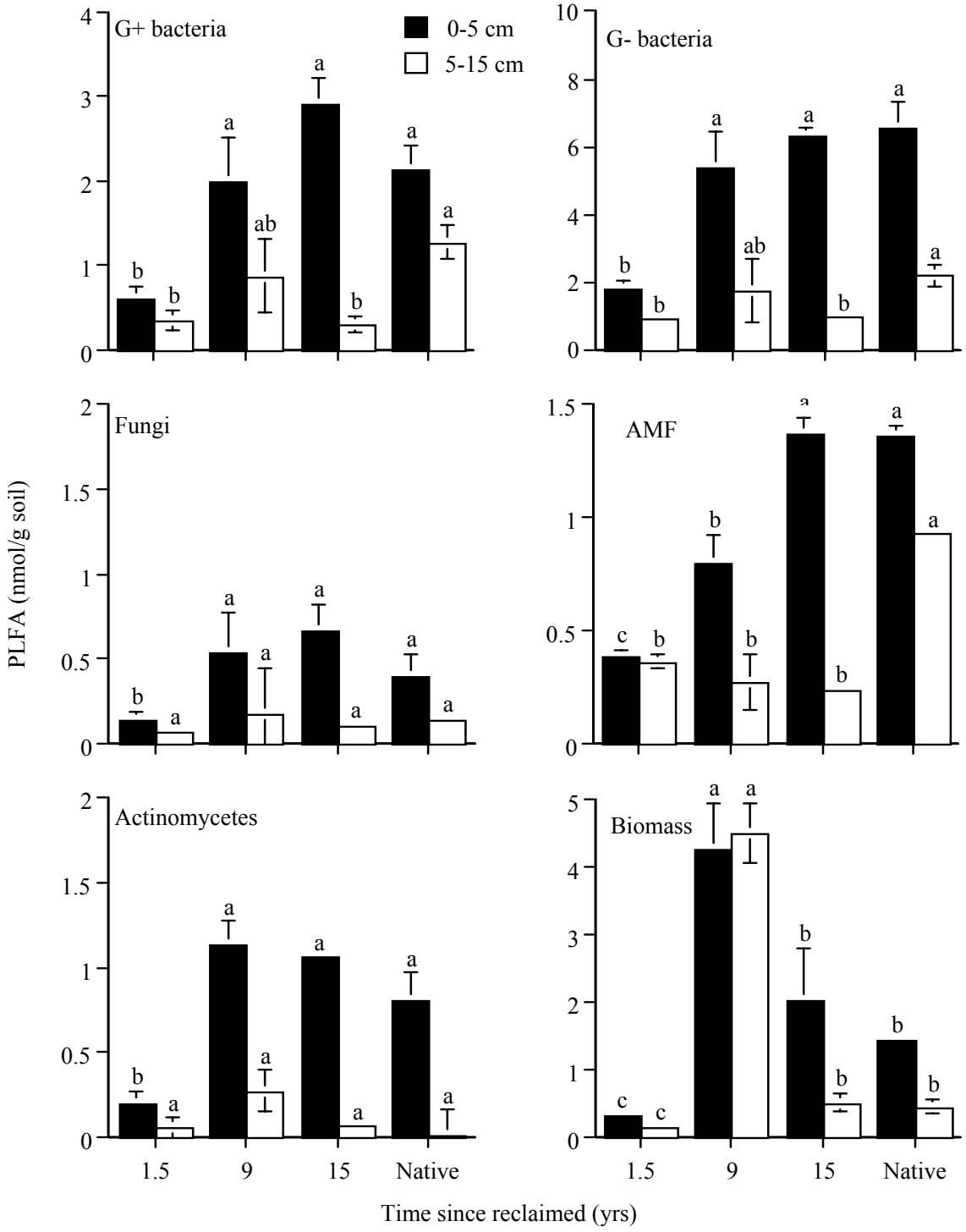

Figure 6. Amounts of various microbial groups (AMF, abuscular mycorrhizal fungi) present in the 0-5 and 5-15 cm soil depths along a chronosequence of sites reclaimed with Stockpiled topsoil and reseeded with cool-season grasses. Sites in located on the Belle Ayr Mine, located in the central Powder River Basin, Wyoming. Lower case letters that are different across reclamtion age are significantly different at $P \leq 0.05$. Bars represent $\pm$ 1 S.D.

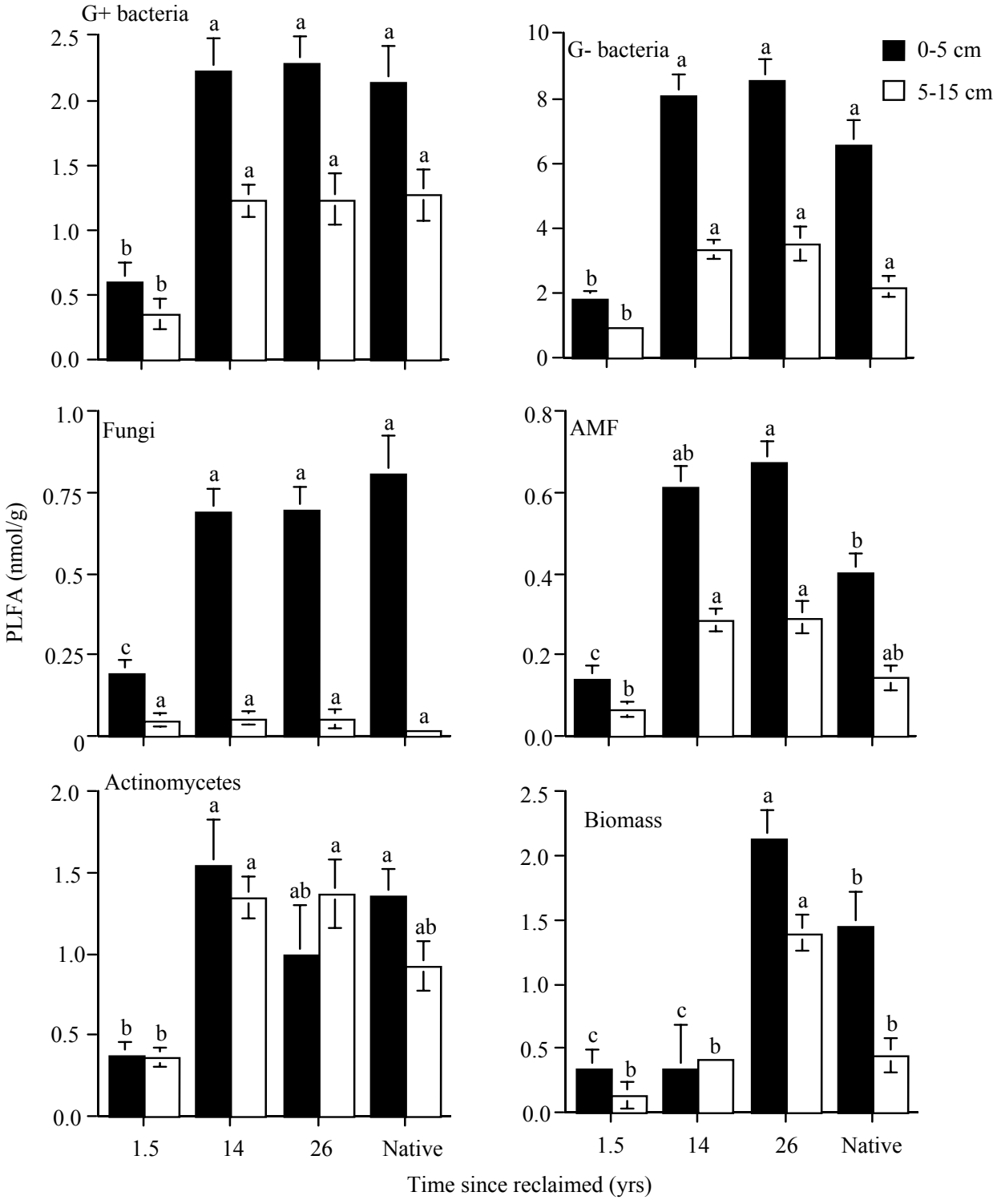

Fig. 7. Amounts of various microbial groups (AMF, arbuscular mycorrhizal fungi) present in the 0-5 and 5-15 cm soil depths along a chronosequence of sites reclaimed with Directly Hauled topsoil and reseeded with cool-season grasses. Sites in located on the Belle Ayr Mine, located in the central Powder River Basin, Wyoming. Lower case letters that are different across reclamation age are significantly different at $P \leq 0.05$. Bars represent $\pm$ 1 S.D.

Greenhouse Experiment

The results of the greenhouse experiment gave no clear indication as to what, if any affect, different plants may have on both microbial community structure or soil aggregation. The soil used in this experiment was obtained from the closed Rosebud Coal Mine, located near Hanna, Wyoming. The soil obtained from there was fairly typical for many soils being of loam texture and having a neutral pH and low electrical conductivity. Soil organic C and total N were, however, quite high leading us to suspect that coal contamination may have occurred.

Table 2. Initial physiochemical properties of the soil used for the greenhouse experiment (T0) as well after 6 months (T6) of being grown with blue grama (BG), cheatgrass (CG), Wyoming big sagebrush (SB) and western wheatgrass (WW)

	pH	EC μS/cm	Texture	OC %	TN %
T0	7.24	378	Loam	3.34	0.167
T6-BG	-	-	-	3.12	0.155
T6-CG	-	-	-	3.17	0.158
T6-SB	-	-	-	3.07	0.160
T6-WW	-	-	-	3.21	0.163

In an attempt to "replicate" the removal, handling, return, and reseeding processes and the impact this has on the breakup of soil aggregates, all the soil used for the greenhouse experiment was sieved to <1mm. The impact this had to break up macroaggregates (Fig. 8) leading to an increase in microaggregates. We would have expected over time however that with plant growth and root production that there would have been a gradual increase in macroaggregate formation and a subsequent decline in microaggregates as they became incorporated into macroaggregates. We did not however see this and we are unsure as to why in fact microaggregates continued to increase and correspondingly macroaggregates continued to decline. A partial explanation may be that due to the nature of the sieved soil (lack of pore space, structure, possible low amount of

organic matter), it was necessary to keep the soils damp throughout the course of the experiment (if they weren't kept moist they became extremely hard and resulted in the death of the seedlings). Previous research has shown that wetting and drying events

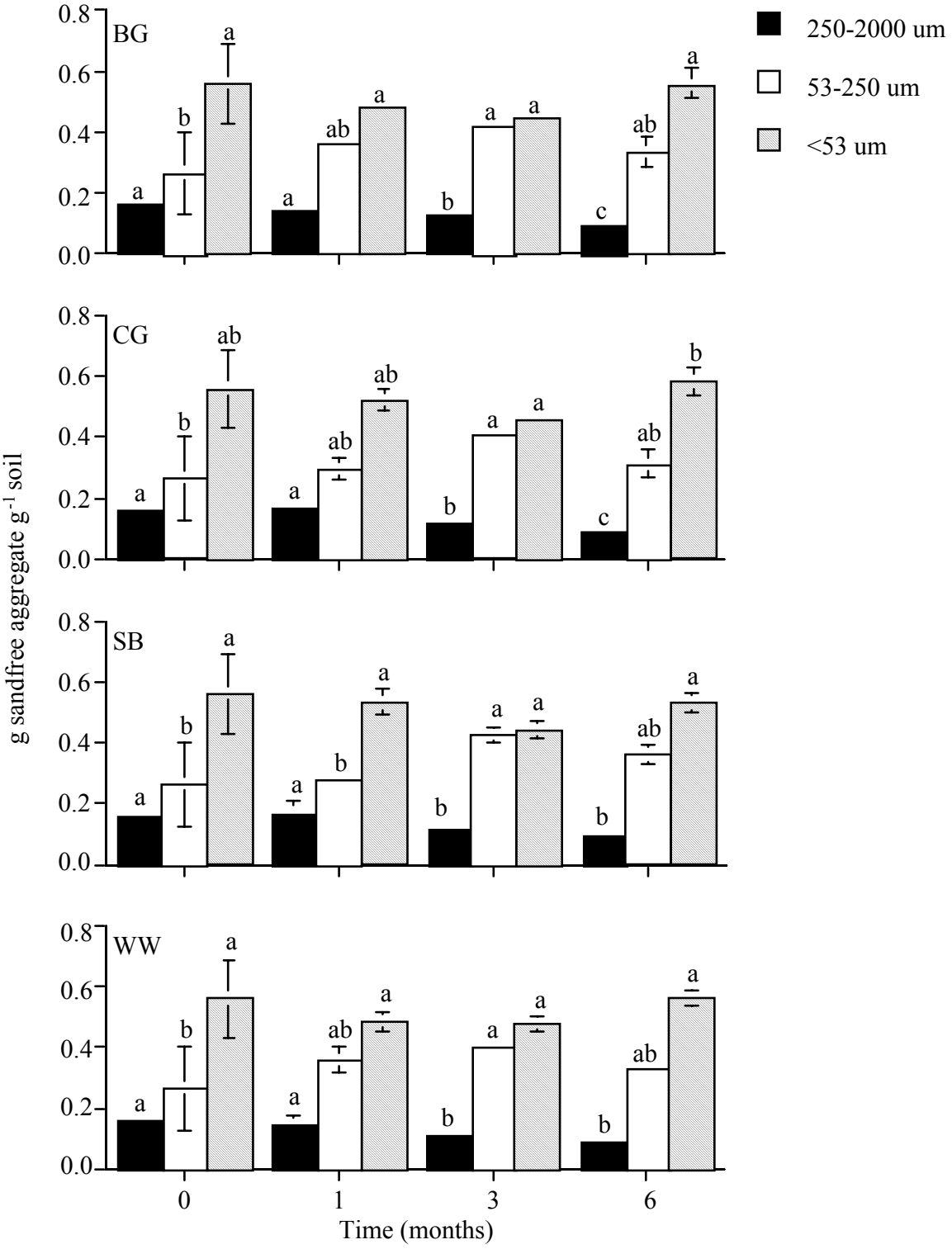

Figure 8. Macroaggregate (250-2000 μm), microaggregate (53-250 μm), and the silt and clay fraction (<53 μm) for soils seeded with blue grama (BG), cheatgrass (CG), Wyoming big sagebrush (SB), and western wheatgrass (WW). Lower case letters that are different across time periods are significantly different at $P \leq 0.05$. Bars represent $\pm$ 1 S.D.

which are common in the field, play an important role in the development of aggregation, and that the need to keep the soils moist over the course of this experiment may have played a role in the inhibition of macroaggregate formation. It is also likely that a period of time, longer than the six months that this experiment ran for, is required for macroaggregates to form.

We had originally planned to measure organic carbon and total nitrogen in the various soil fractions (light fraction, heavy fraction, and silt and clay) for each of the four plant species at the completion of the experiment however our measurements of organic

Table 3. Proportion and concentration of organic carbon and nitrogen in light fraction (LF), heavy fraction (HF), and silt and clay (SC) concentrations of carbon and nitrogen in the soils used for the greenhouse experiment (GH) as well as ranges recorded in reclaimed (Rec.) and native (Nat.) soils.

Site	**Fraction**	**LF**	**HF**	**SC**			
		g sand corrected aggregate g^{-1} soil					
GH	250-2000 μm#	0.134	0.212	0.595			
	53-250 μm	0.046	0.232	0.721			
		% C-LF	**% C-HF**	**% C-SC**	**%N-LF**	**%N-HF**	**%N-SC**
GH	250-2000 μm	32.7	0.360	1.82	1.33	0.027	0.119
	53-250 μm	37.5	0.387	1.27	1.39	0.032	0.107
Rec.*	250-2000 μm	10.9-27.1	0.16-1.5	0.66-2.3	0.67-1.10	0.05-0.13	0.08-0.20
	53-250 μm	25.2-40.9	0.10-0.94	0.84-2.2	0.94-1.50	0.03-0.08	0.18-0.75
Nat.†	250-2000 μm	13.9-15.5	0.39-0.52	2.1-2.9	0.95-1.20	0.03-0.05	0.22-0.28
	53-250 μm	14.9-17.9	0.35-0.81	1.7-2.3	0.19-0.23	0.24-0.69	0.19-0.23

#May not add to 1 due to sand present within macroaggregates
* Ranges for macroaggregate and microaggregate concentrations Reclaimed site are ranges from sites varying in age from <1 to 26 years
† Native site ranges were determined from 2 native sites sampled at the Belle Ayr Mine

carbon in these fraction that there was such a high level of coal carbon that it would have simply swamped any increases that may have been observed due to plant species (Table 3). As can be seen in Table 3, the values we recorded where generally much higher than that found in various fraction in both undisturbed native soils as well as reclaimed soils.

Microbial community structure:

There was generally little influence of plant species on microbial community structure (Figure 9). The most obvious changes were: 1) a decline in actinomycetes for species over time; 2) an increase in protozoa in blue gram and cheatgrass towards the end of the experiment, and; 3) a general decline in the Bacteria:Fungal ratio. The decline in actinomycetes was also observed in the filed over time but over a much longer span of time. The increase in protozoa suggests that they were probably present in the soil in very small quantities but only in the blue grama and cheatgrass soils were conditions conducive for their growth after lying dormant for three to six months. We are at a loss to explain why there was a decline in the Bacteria:Fungal ratio as we would have expected to increase over time. It is most likely explained by the short duration of this experiment not allowing sufficient time for fungi to become established.

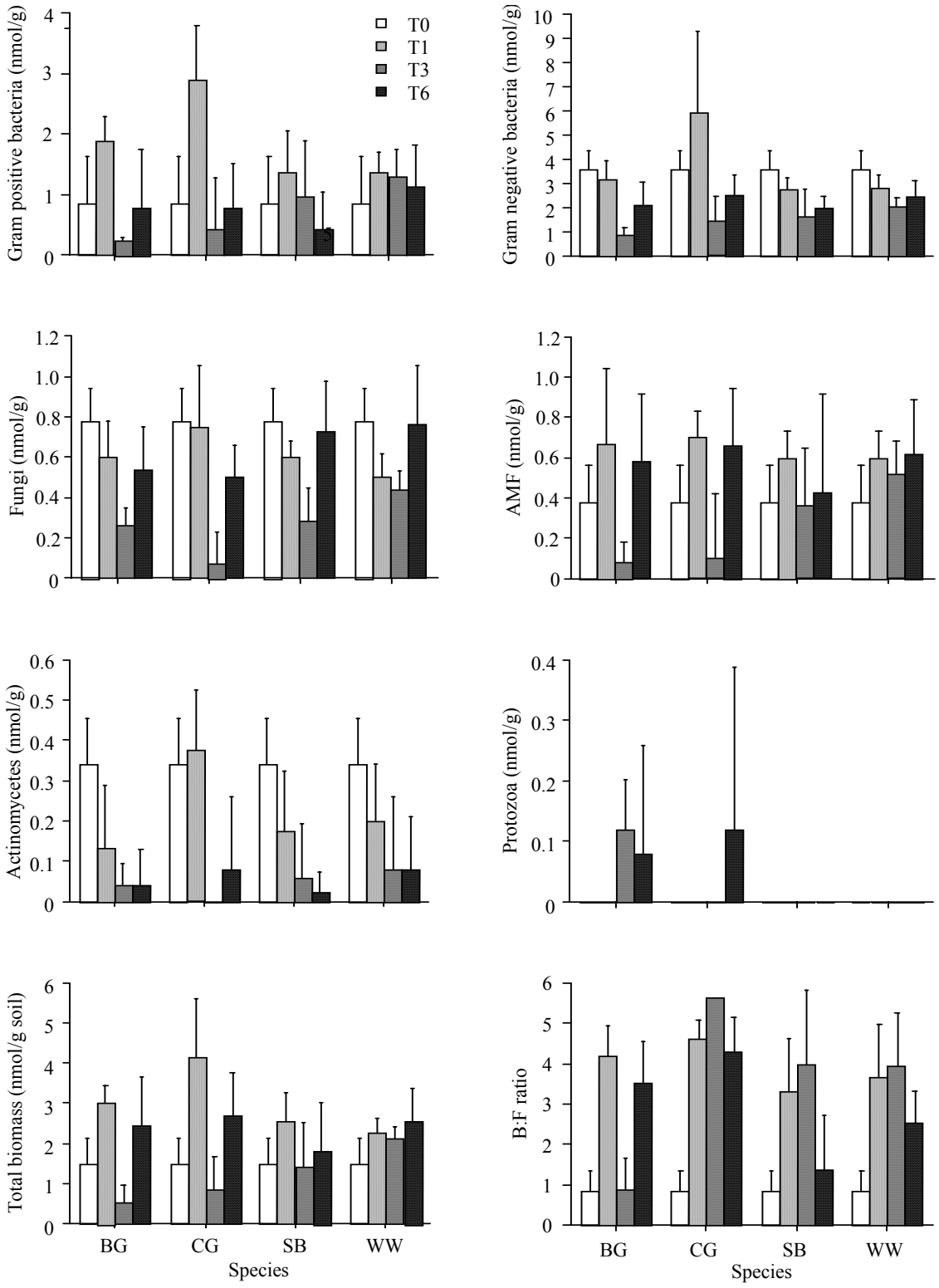

Figure 9. Amounts of various microbial groups (AMF, arbuscular mycorrhizal fungi) measured at 0 (T0), 1 (T1), 3 (T3), and 6 (T6) months after germination in soils planted to blue grama (BG), cheatgrass (CG), Wyoming big sagebrush (SB), and western wheatgrass (WW). Bars represent $\pm$ 1 S.D

Management Implications

- Stockpiling of soils leads to a further decline in aggregation compared to the direct hauling of soils (though this may be the result of soil textural differences). This will lead to a loss of nutrients and carbon and in the short term will result in a lower quality growth media for seeded plant communities.
- Possibly because of a combination of greater aggregation and more stable aggregate formation in the directly hauled soils, new carbon inputs from plant litter (above- and below ground) carbon is being held in aggregates and is thus better protected from microbial decomposition and losses to microbial respiration. This will have the effect of increasing soil organic matter, nutrients, water-holding capacity, bulk density, porosity, and cation exchange capacity; all of which will improve soil quality for new plant growth.
- No difference in most microbial groups between directly hauled and stockpiled soils after approximately 15 yrs. This suggests that microbial communities have recovered to the extent they are now similar to native soil microbial communities. We are unable to determine how old sites need to be to have "recovered" (and it will depend on a range of factors, i.e. plant community type, initial soil quality, climate, etc) but appears to be in the time scale of of 5-10 yrs. Fungi appear to take the longest to recover
- The use of soil organic carbon as a general indicator of soil 'quality' must take into consideration the strong possibility of coal present in soil aggregates (and thus not readily visible) can likely artificially inflate estimates of soil organic carbon/organic matter.