SOIL AGGREGATE AND AGGREGATE ASSOCIATED CARBON RECOVERY IN SHORT-TERM STOCKPILES[1]

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Abstract: Soil organic matter (OM) is drastically reduced by various processes (erosion, leaching, decomposition, dilution through soil horizon mixing etc.) typically associated with topsoil salvage prior to surface mining activities. Of these processes, loss of physical protection of OM through the breaking up of soil aggregation can result in up to 65% of soil carbon (C) reductions. Objectives of this research were to monitor soil aggregate size distribution and associated C throughout short-term stockpiling and subsequent utilization of topsoil for reclamation. Soil samples were collected from the top 5 cm of a stockpile over a 3 year period (<1, 1.5, 3 yrs) and an adjacent undisturbed, native site. Surface stockpile soils were then tracked to a temporary location following stockpile removal and sampled again. Samples were analyzed for aggregate size distribution, fractions, associated C, and OM turnover with ^{13}C natural abundance. Macroaggregation increased and microaggregation decreased after 3 yrs of storage, indicating recovery of aggregation in 3 yrs. Following the second removal, macroaggregate proportions decreased and silt and clay fractions were greater than that observed in the native site soils. The second disturbance resulted in greater destruction of aggregate structure than the initial disturbance during topsoil salvage. Carbon increased significantly between <1 and 1.5 yrs in both aggregate size classes. Macro- and microaggregate light fraction (LF) C decreased with storage time as this material was available for utilization by microbes. Aggregate δ^{13}C values indicated up to 65% new C associated with aggregate structure. Topsoil storage was beneficial for aggregation and associated C in the surface layers only where plant roots and microbial communities are active; however, the second movement of the topsoil resulted in loss of soil aggregation without impact to soil C.

Additional Key Words: Isotope, cool season grass, disturbance, mining, coal.

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Introduction

Surface coal mining regulations require topsoil salvage from areas prior to mining and reuse in reclaimed areas during reclamation efforts (SMCRA, 1977). Under this regulation, salvaged topsoil is either stored in stockpiles or is directly hauled to areas undergoing reclamation. In the 1980's and early 1990's, properties of stockpiled soils were studied to gain an understanding of the impacts of storage on topsoil material. More specifically, research focused on soil microbial community structure, chemical (pH, nitrogen) and physical properties (bulk density) with minimal work investigating soil aggregation and organic matter (OM) dynamics (Vogel, 1987). Losses of aggregation with topsoil stripping and storage have been thought to contribute greatly to declines in soil OM (48% of which is C; Brady and Weil, 2002) in reclaimed systems (Ingram et al., 2005). It has been generally stated that aggregation is slow to recover (Visser et al., 1984; Harris et al., 1989) and whole soil carbon (C) is greatly reduced (Abdul-Kareem and McRae, 1984; Visser et al., 1984; Ghose, 2004) in all soil depths with stockpiling. Variable results concerning recovery of stockpiled soil properties toward native soil conditions with restoration have been reported. Soil C and microbial biomass did not reach levels found in native soil in colder climates of Alberta or wetter climates in the UK (Insam and Domsch, 1988; Williamson and Johnson, 1990; Johnson and Williamson, 1994); however, have been found to exceed native soil conditions in semi-arid environments (Rana et al., 2007; Wick, 2007). The impact of storage on these soil properties is dependent upon methods of topsoil stripping and storage as well as the amount of time in storage (Visser et al., 1984).

Soil aggregation, more specifically macro-(250-2000 μm) and microaggregates (53-250 μm), directly influences soil properties such as, but not limited to, bulk density, pore size distribution (Hillel, 1982), microbial community structure (Paul and Clark, 1996), and soil resistance to erosion (Valmis et al., 2005). Soil OM indirectly contributes to soil structure by serving as a nucleus for aggregate formation (Six et al., 1998). Aggregation is also closely tied with soil function by physically protecting OM and is therefore a regulator of microbial decomposition and nutrient availability for plant uptake (Essington, 2004). Significant loss of OM generally results from topsoil stripping and/or storage prior to mining as a result of increased microbial decomposition rates and dilution of OM through soil horizon mixing (Visser et al., 1984; Ussiri and Lal, 2005; Lorenz and Lal, 2007). Also, based on observations in agricultural systems and previous reclamation research, most macroaggregation is destroyed during topsoil stripping and salvage prior to mining as roots and fungal hyphae holding macroaggregates together are disrupted (Wick, 2007). Soil OM has been found to increase with reclamation age, but there is not always a corresponding increase in soil macroaggregation (Malik and Scullion, 1998). However, we hypothesize recovery of aggregation in reclaimed soils, even after short-term stockpiling, could be an important mechanism for OM accumulation (Ingram et al., 2005; Wick, 2007). An understanding of aggregates and aggregate associated OM in stockpiled soils prior to replacement on reclaimed areas is necessary for the interpretation of reclaimed soil dynamics.

Though the relationship between OM and aggregates is important, mechanisms of OM incorporation into soil aggregates is a poorly understood component of soil recovery from massive disturbances such as surface mining. Stable C isotopes can be used to track the incorporation of "new" OM in soil aggregates following a land use conversion if the plant community has changed from warm season (C_4) grasses ($\delta^{13}C$ of -12 to -16‰) to cool season (C_3) grasses ($\delta^{13}C$ of -25 to -29‰), or vice versa (O'Leary, 1988). Many studies have used this technique to observe OM incorporation, dynamics and aggregate hierarchy in agricultural or land use change soils (Jastrow et al., 1996; Six et al., 2000; Lobe et al., 2005; Schwendenmann and Pendall, 2006). Mining disturbances in the semiarid west often result in a change in plant community composition. Undisturbed, northern mixed grass plant communities found in Northeastern Wyoming consist of approximately 70% C_3 and 30% C_4 plants compared to the seeded vegetation used to stabilize stockpiles which typically contain 100% C_3 plants (Wick et al., 2007). Utilizing stable C isotopes to understand OM incorporation and dynamics in reclaimed systems will increase our understanding of C cycling in stockpiled and reclaimed soils.

Objectives of this research were to: (1) quantify aggregate proportions through time in stockpiled soils, (2) quantify organic C in the macro- and microaggregate size classes, (3) quantify and determine the location (light, heavy, and mineral fractions) of new OM within each aggregate size class, and (4) observe changes in the above properties with a second disturbance or topsoil stockpile utilization for reclamation. Objectives will be accomplished through repeated measures of a single stockpile rather than across stockpile sites of varying ages. We hypothesize a greater abundance of macroaggregates and a minimal increase in C with short-term stockpile storage, soil macroaggregate $\delta^{13}C$ values will be most similar to roots of vegetation seeded on the stockpiles for stabilization, and microaggregate $\delta^{13}C$ will remain similar to the native soil signatures.

Materials and Methods

Site Information and Field Sampling

A topsoil stockpile (covering 22 ha, 18 m at the highest point) was sampled at <1, 1.5, 3 yrs and following removal and replacement to a temporary location (<1 Removed) at the Belle Ayr Coal Mine in the Powder River Basin, Wyoming, USA (Figs. 1 and 2). An adjacent undisturbed, native site was also sampled to determine aggregate size distributions and C pools of unperturbed soil. Average annual precipitation for the area is 376 mm and mean annual air temperature is 6.7°C (Belle Ayr Mine, 2005). Cool season grass species growing on the stockpile during the 1.5 and 3 yr sampling times consisted of [*Pascopyrum smithii* (Rydb.) A. Löve, *Nassella viridula* (Trin.) Barkw., and *Hesperostipa comata* (Trin. & Rupr.) Barkw.]. Minimal vegetation was observed for the <1 yr sampling and no vegetation for the <1 Removed sampling time. Vegetation at the native site was dominated by *Pascopyrum smithii* (Rydb.) A. Löve, *Nassella viridula* (Trin.) Barkw., *Hesperostipa comata* (Trin. & Rupr.) Barkw, *Bouteloua Gracilis* (H.B.K.) Lag. ex. Steud., and *Artemisia tridentate* var. Wyomingensis (Beetle and Young) Welsh. Prior to disturbance, soils were classified as coarse-loamy, mesic Ustic Haplargids (Munn and Arneson, 1999).

Figure. 1. Digital image of Belle Ayr Mine, Gillette, Wyoming from 30,000 feet. Site locations indicated with red star. Source: Google Earth.

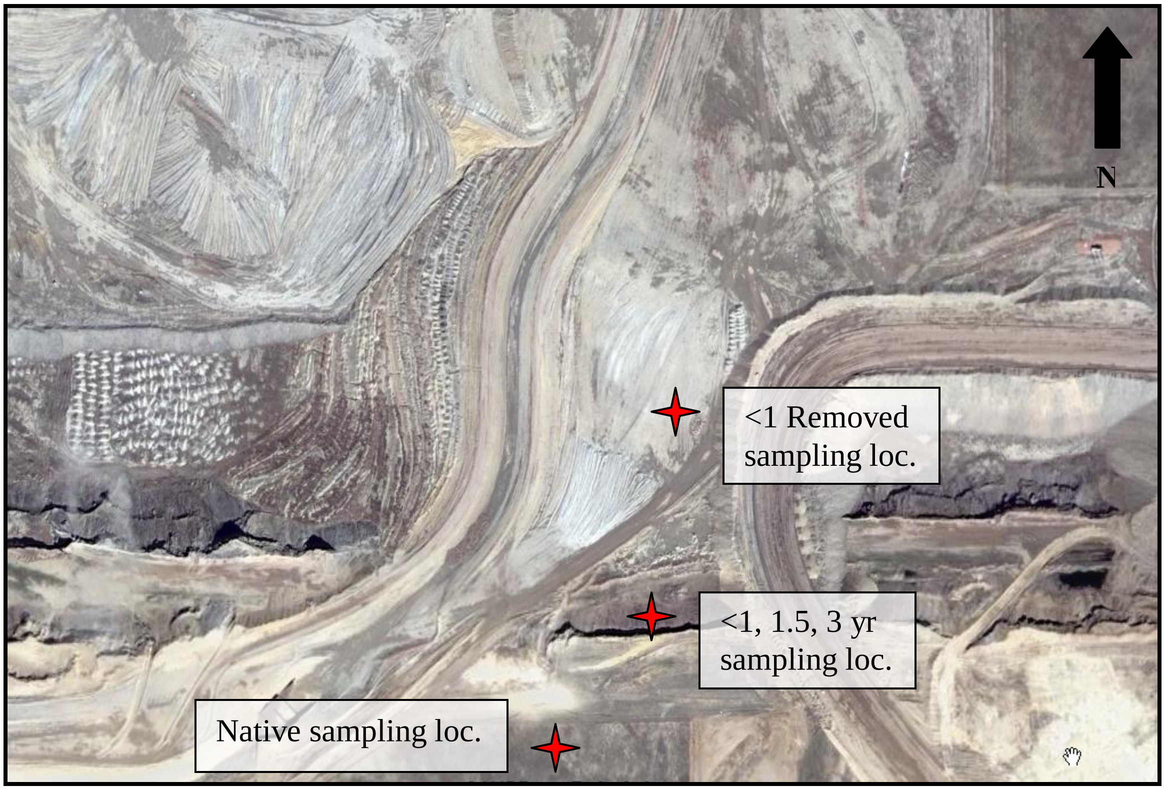

Figure. 2. Close up of Belle Ayr Mine, Gillette, Wyoming where stockpiled samples were collected. Sites indicated with red stars. Image taken from 10,000 feet. Source: Google Earth.

Soil samples from the surface of the stockpile were collected in August, 2005 (<1 yr), May, 2006 (1.5 yr), July, 2007 (3 yr) and October, 2007 (<1 Removed) (Figs. 3-6). The <1 yr sampling occurred within 3 months of stockpile seeding and <1 Removed sampling was conducted immediately following topsoil placement on the new site (within 2 days). The native site was sampled in May, 2006 (Fig. 7). The top 5 cm of soil were collected with a trowel at four points along each of three randomly oriented, 45 m transects. From the native site, 5-15 and 15-30 cm depths were also collected with a 2.5 cm diameter step probe along the same transects to get a better representation of native topsoil conditions prior to stripping and storage (which ranges from 15 to 60 cm of material salvaged during topsoil removal). Samples were collected from the same transects for the first 3 sampling times on the stockpile. Previously sampled soils from the top 5 cm of the stockpile were tracked to a temporary location and resampled along three new 45 m transects. Five samples were collected for bulk density (BD) at each site using a double cylinder, hammer driven core sampler (Grossman and Reinsch, 2002). Root samples were collected from the 1.5 yr old stockpiled soil.

Figure. 3. Stockpile at <1 yr sampling time.

Figure. 4. Stockpile at 1.5 yr sampling time.

Figure. 5. Stockpile at 3 yr sampling time.

Figure. 6. Stockpiled soils following removal.

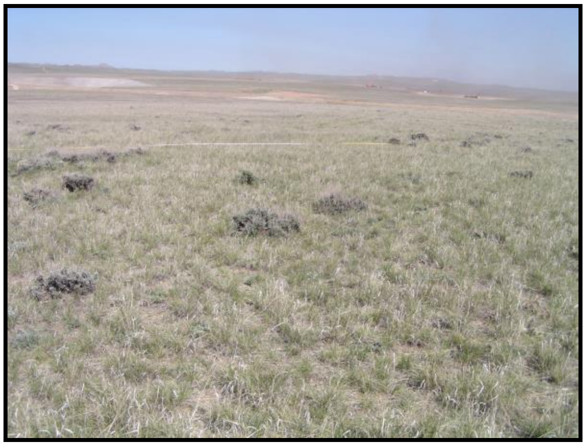

Figure. 7. Native site vegetation.

General Soil Properties

Samples were air dried and dry sieved to 2000 µm to remove large roots and break apart soil clods while leaving structure <2000 µm intact. Particle size distribution was determined on a subset of samples (4 per site) from the <1 yr stockpile sampling time and native soil using the hydrometer method (Gee and Or, 2002). Electrical conductivity (EC) and pH were determined on a 1:1 soil:water mixture. An Oakton con 100 series EC probe (Vernon Hills, IL) and a Fisher Scientific Accument Basic pH meter with a glass electrode (Pittsburgh, PA) were used for analysis, EC and pH respectively. Total soil C and N values were obtained by dry combustion with a Carlo Erba NC 2100 Analyzer (Lakewood, NJ). Inorganic C (IC) content of soil was measured with the modified pressure calcimeter method (Sherrod et al., 2002). Organic C content was quantified by subtracting IC from total C.

Aggregate Size Distribution

Water stable aggregate size distributions of soil were determined using a wet sieving protocol described by Six et al. (1998). In summary, 100 ± 0.02 g of air dried soil were submerged in deionized water for 5 min at room temperature on a 250 µm sieve. Water stable macroaggregates (250-2000 µm) were separated from the whole soil by moving the sieve 3 cm up and down 50 times in 2 min. Material (water plus soil) that passed through the sieve was transferred to a 53 µm sieve and the above process 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.

Sand is the same size as macro- and microaggregates; therefore, it was necessary to correct for sand content across sites. Sand corrections were determined for a subset of samples (4 samples per site) according to Denef et al. (2001). Five mL of sodium hexametaphosphate and 10 mL of water were added to separate 5 gram subsamples of macroaggregates and microaggregates. Samples were shaken on a reciprocal shaker for 18 h 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).

Density Floatation

Particulate OM (POM) analysis was conducted on all samples according to methods described by Six et al. (1998). Samples of macro- and microaggregates (8 grams) were oven dried overnight at 105˚C. The samples were 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 the 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 10 min to remove air trapped within aggregates. Samples were centrifuged for 60 min at 2,500 rpm and floating material (light fraction, LF) was aspirated through a 20 µm nylon filter and rinsed with deionized water. The material on the filter was transferred into a beaker and dried at 55˚C overnight. The material remaining in the centrifuge tube (intra-aggregate POM (iPOM), sand, silt and clay) was rinsed twice with deionized water, flocculated with 5 drops of 0.25 M $CaCl_2$ and 0.25 M $MgCl_2$ and centrifuged at 20˚C for 15 min at 2,500 rpm. Twelve 6 mm glass beads were added to each centrifuge tube, which were then placed on a reciprocal shaker for 18 h. Samples were removed from the shaker and sieved with nested 250 and 53 µm sieves for macroaggregate samples and a 53 µm sieve for microaggregate samples. Material remaining on the sieve (iPOM and sand, termed heavy fraction, HF) and material washed through the sieve (Mineral) were dried at 55˚C overnight.

Aggregate Associated Carbon and Isotopic Analysis

Samples (macro- and microaggregates, aggregate fractions, and roots) from the 0-5 cm depth of all sites were analyzed for organic C using dry combustion on a Carlo Erba NC 2100 Analyzer (Lakewood, NJ) and for ^{13}C with a Thermo Finnigan Delta Plus XP Continuous Flow Stable Isotope Ratio Mass Spectrometer (Bremen, Germany). Additional samples (5-15 and 15-30 cm depths) were analyzed for C and ^{13}C for the native soil to get a better representation of the mixed soil used during reclamation. Weighted averages were calculated based on C concentration and isotopic signature for each depth (Jastrow, personal communication). Inorganic C was not detected in the whole soil or aggregate samples so total C was considered organic C. Comparisons of C concentrations across sites are not valid unless corrected for sand (Elliott et al., 1991). The following formula was used to calculate the sand free C content (Equation 1) for each size class (Denef et al., 2001):

$$\text{Sand free } C_{\text{fraction}} = C_{\text{fraction}} * [\text{g aggregate}_{\text{fraction}} / (1 - HF_{\text{proportion}})] \qquad (1)$$

Percentage of new C addition since disturbance was calculated for the stockpiled soils of different ages using a simple mixing model (Equation 2) (Six et al., 2001):

$$\%C_{new} = [(\delta^{13}C_{(stockpile\ at\ different\ ages)} - \delta^{13}{}_{native})/(\delta^{13}C_{roots} - \delta^{13}C_{native})] \times 100 \qquad (2)$$

Where $\delta^{13}C_{\text{(stockpile at different ages)}}$ = $\delta^{13}C$ of OM in bulk and aggregate fraction in stockpiled soils of different ages (<1, 1.5, 3, <1 Removed), $\delta^{13}C_{native}$ = mean $\delta^{13}C$ of SOM in bulk and aggregate fraction in native soils (used as an endpoint), and $\delta^{13}C_{roots}$ = $\delta^{13}C$ of fine root material for stockpiled sites ($\delta^{13}C$ = -22.86 ‰).

Statistical Analysis

Statistical analyses were conducted using JMP version 4.0.4 (JMP, 2001). Data were analyzed with one way analysis of variance across stockpile ages to determine differences in soil properties after varying amounts of time in the stockpile. Student's t-tests were used to determine significance between groups following analysis of variance. All statistical tests were conducted at $P<0.05$ with exception of whole soil C and N which were tested at $P<0.10$.

Results and Discussion

General Soil Properties

The stockpiled topsoil was clay loam in texture (39% sand, 34% silt, 27% clay) and the native soil was a sandy clay loam (53% sand, 26% silt, 21% clay). Bulk density was lower (1.26 g cm^{-3}) for the surface of the stockpile in the first year of sampling compared to the 1.5 yr (1.49 g cm^{-3}), 3 yr (1.41 g cm^{-3}) and second movement of the topsoil (1.60 g cm^{-3}) sampling times (Table 1). Only the second movement of the topsoil (<1 Removed) BD was significantly higher (20%) than the native surface soils (1.34 g cm^{-3}). Abdul-Kareem (1984) also observed a 20% increase in BD of clay loam stockpiled soils compared to a native undisturbed site. Electrical conductivity was significantly higher in <1 yr and <1 Removed sites (0.05 and 0.06 S m^{-1}, respectively) compared to other sampling times and all stockpiled soils had significantly higher EC values than the native soil (0.02 S m^{-1}). Soil pH was also higher for stockpiled soils (7.32 – 7.83) compared to the native surface soils (6.61). These differences in stockpiled and native soils are probably due to the mixing of soil horizons during removal and stockpiling.

General soil properties do not appear to be influencing aggregate formation in the stockpiled soils (i.e. clay content is relatively high, compaction is minimal, salts are low and pH is not limiting for microbial activity); however, comparisons between the native and stockpiled sites could be affected by the difference in soil texture. Significantly higher clay content of the stockpiled soils (27%) could contribute to greater aggregation through the reorientation of clay particles and binding of root exudates to clay by wet-dry cycles compared to the native site with lower clay content (21%) (Reid and Goss, 1982).

Table 1. General soil properties for stockpile (<1, 1.5, 3 and <1 Removed) and native sites at Belle Ayr Mine, Gillette, WY. Properties include electrical conductivity (EC), pH, bulk density (BD), soil organic carbon (SOC), nitrogen (N), and carbon:nitrogen ratio (C:N). Significance shown across sites at P<0.05 for EC, pH and BD and P<0.10 level for SOC and N.

Site Age	**EC**	**pH**	**BD**	**SOC**	**N**	**C:N**
(yrs)	(S m^{-1})		(g cm^{-3})	(Mg ha^{-1})	(Mg ha^{-1})	
Native	0.02 c	6.61 c	1.34 b	9.92 a	0.958 a	10:1
<1	0.05 a	7.83 a	1.26 b	5.59 bc	0.467 b	12:1
1.5	0.03 b	7.77 a	1.49 ab	5.09 c	0.489 b	10:1
3	0.04 b	7.62 a	1.41 ab	5.63 bc	0.536 b	11:1
<1 Removed	0.06 a	7.32 b	1.60 a	6.13 b	0.549 b	11:1

Whole soil C remained unchanged throughout stockpile age; however, significantly increased following removal of the stockpile and relocation to another site (Table 1). All stockpile C values were significantly lower than the native soil concentrations primarily as a result of dilution through topsoil and subsoil mixing and also some microbial decomposition with the increase in oxygen availability during removal and handling (Visser et al., 1984; Ussiri and Lal, 2005; Lorenz and Lal, 2007). Soil N remained unchanged with stockpile age and was lower than the native soil concentrations in the surface of the stockpile. Carbon:Nitrogen ratios were similar throughout time in the stockpile.

Aggregate Size Distribution

Aggregate size distribution at the time of the <1 yr sampling was typical of disturbed soils, with high microaggregation and relatively low macroaggregation (Six et al., 1998) (Fig. 8). There was a minimal, but significant increase in macroaggregate stability (which may indicate a trend towards recovery) between the recent placement (<1 yr) and 3 yrs of topsoil storage from 0.18 to 0.26 g macroaggregate g^{-1} soil, respectively. Though this level of macroaggregation is still very low compared to native tallgrass prairie soils observed by Jastrow (1996) and Six et al., (1998); this is greater than that observed in the native soils in this area (0.06 g macroaggregate g^{-1} soil). Previous research by Gee and Bauer (1976) found no change in aggregate stability with stockpile storage time, while Abdul-Kareem and McRae (1984) reported aggregate stability decreases with stockpile age. We hypothesize aggregate recovery trends in this study are the result of soil biological activity stimulated by the seeding of vegetation (a practice initiated in the mid 1980's) in the surface of the stockpile.

Microbial secretions and fungal hyphae have generally been found to be extremely important components of macroaggregate development (Six et al., 2004). Previous stockpile studies have extensively identified influences of stockpiling on soil biological parameters. Barkworth and Bateson (1964) found bacterial populations decreased following stockpiling in Britain and other observations by Ross and Cairns (1981), Abdul-Kareem and McRae (1984) and Harris and Birch (1987) indicated large decreases in soil microbial biomass following initial stripping and storage of topsoil. Visser et al. (1984) observed decreases in microbial C in stockpiled soils with time with little change to decomposition potential and primary production in topsoil stored for <3 yrs. General decreases in microbial biomass should inhibit the formation of soil macroaggregates with stockpile storage time, but was not observed in this study.

Following relocation of the topsoil stockpile and placement in a temporary location, macroaggregates decreased (0.19 g macroaggregate g^{-1} soil) to levels observed in the <1 yr sampling (0.18 g macroaggregate g^{-1} soil), microaggregate proportions continued to decrease further (0.45 g microaggregate g^{-1} soil) and silt and clay proportions increased from 0.26 in the 3 yr sampling to 0.36 g silt and clay g^{-1} soil (Fig. 8). Aggregate size distributions following the second topsoil transport were similar to those observed in the undisturbed, native sandy clay loam soils. Increases in proportions of silt and clay with less aggregation had great effected on, but not limited to, soil bulk density, and could therefore impact water infiltration and air movement (Hillel, 1982). It is clear that regardless of macroaggregation recovery with stockpile storage time, this gain in structure is subsequently lost following the second topsoil movement and broken apart into even finer material (silt and clay) compared to the initial disturbance. It is also possible that newly formed aggregates (<3 yrs old) are more susceptible to breaking apart than those developed in undisturbed soil.

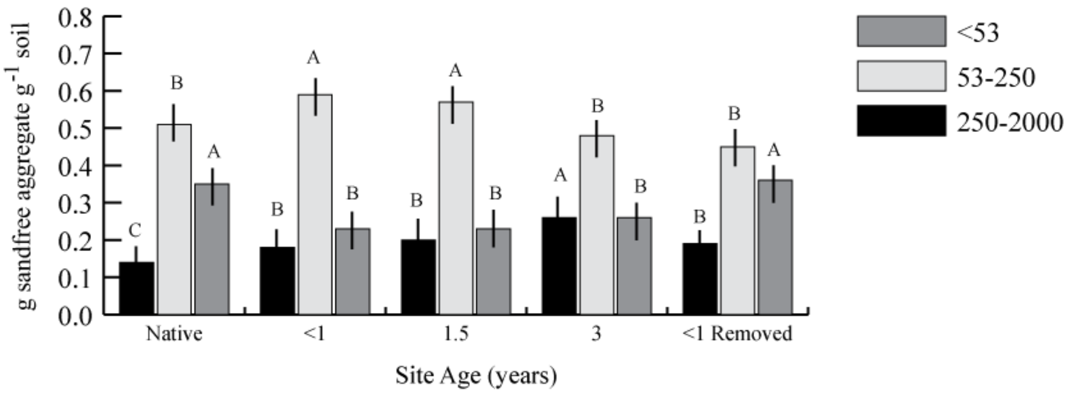

Figure 8. Aggregate size distribution for the 0-5 depth of stockpiled and native soils at Belle Ayr Mine, Gillette, WY. Significance shown across site ages for macroaggregates (250-2000 µm), microaggregates (53-250 µm) and silt and clay (<53 µm) at the $P<0.05$ level. Bars represent standard deviation, n=12.

Field studies on aggregate development in the short-term are lacking. Indications that stable macroaggregates formed in <3 yrs is important for understanding the recovery of physical properties following a disturbance. With macroaggregate formation in a short period of time, surface stockpiled soil physical properties are improving in the short-term to possibly lead to improved biological properties. Soil heterogeneity created by various sizes of aggregates provides protection for bacteria from predators, protects a crucial energy source (OM) for bacterial populations, increases and allows water and roots to infiltrate and penetrate into soils to a greater depth (Paul and Clark, 1996; Six et al., 2004). Improvement to all these properties in the surface soils of a stockpile is important to the redevelopment of disturbed soils. It is possible that long-term storage of topsoil (rather than the short-term storage observed in this study) may result in more stable aggregated topsoil to a greater depth (>5 cm) upon replacement during reclamation. It is, however, important to recognize that because of the size of stockpiles, the surface of the stockpile amounts to only a small portion of soil used during topsoil replacement on reclaimed areas.

Aggregate Carbon

Both aggregate size classes and aggregate fraction C concentrations were significantly reduced by topsoil stripping prior to mining (Fig. 9), as seen through comparing the native site to the <1 yr sampling time. Macroaggregate C concentrations decreased by 73% and microaggregate C by 63% (Fig. 9a). The greatest percentage loss of C was in the LF for both aggregate size classes (macroaggregate LF: 86%, microaggregate LF: 80%) (Fig. 9c).

Macroaggregate HF C was reduced by 73% and microaggregate HF C by 62% (Fig. 9b). Less change was observed in the macroaggregate Mineral C concentrations, with a 36% reduction. A substantial loss (52%) was still observed in the microaggregate Mineral C (Fig. 9d). Visser et al. (1984) observed similar losses (up to 50%) in whole soil C with topsoil stripping and storage; however, their data indicate C continued to decline in surface soils with time in stockpile for the following 3 yrs after initial placement.

Interestingly, macroaggregate associated C increased significantly in surface soils within 1.5 years of topsoil storage (24.50 to 43.20 g C g^{-1} sandfree aggregate for the <1 and 1.5 yr old sites, respectively) and remained unchanged even following the second topsoil relocation. Microaggregate C increased between the <1 and 1.5 yr old stockpile sampling times from 38.50 to 76.50 g C g^{-1} sandfree aggregate and reached a concentration similar to the native soil following the second topsoil movement (104.3 g C g^{-1} sandfree aggregate). Though there are no other studies measuring aggregate C to this detail for stockpiled soils, these results are contrary to findings by Abdul-Kareem (1984), Visser et al. (1984), Harris and Birch (1987) and Ghose (2004) where whole soil C decreased with storage time (possibly because of depth or lack of vegetation on stockpiled soils in these studies). Minimal change in C concentrations of the soils following the second movement and replacement is consistent with Williamson and Johnson (1990). It is possible microbial activity was again reduced following the second movement of the topsoil resulting in similar aggregate C values after 3 yrs of stockpile storage.

In all soils, C concentrations in microaggregates were greater than that observed in macroaggregates. This phenomenon has not been observed in subhumid tilled soils (Jastrow et al., 1996; Six et al., 1998); however, was a trend observed in other semiarid reclaimed and native soils (Wick, 2007). Higher microaggregate C concentrations in stockpiled soils could have resulted from reduced microbial activity in the stockpiles (Yamashita et al., 2006). Because of the importance of fungal hyphae in macroaggregate stabilization and bacteria in microaggregate stabilization, declines in fungal activity (as observed by Visser et al., 1984), but not bacterial activity, would lead to less decomposition in the macroaggregate size fraction (Mummey et al., 2002), less incorporation of C into chitin (Holland and Coleman, 1987) and therefore the incorporation of OM high in C into microaggregate pools. Mechanical disturbance of soil such as tillage and stripping are well known to impact fungi to a much greater extent than bacteria (Mummey et al., 2002; Stahl et al., 2003)

Though C concentrations were much lower than what would be found in native soils, the small increase within 1.5 yrs of storage still indicated that surface contributions of plant litter and root turnover could potentially create additional pockets of readily decomposable, small pieces of plant litter in the topsoil when replaced during reclamation. Again, because of the size of stockpiles, the surface soils account for a very small portion of the topsoil utilized during reclamation. We do, however, speculate that these pockets of OM could lead to increasing reclaimed soil heterogeneity though variable microbial communities and habitats.

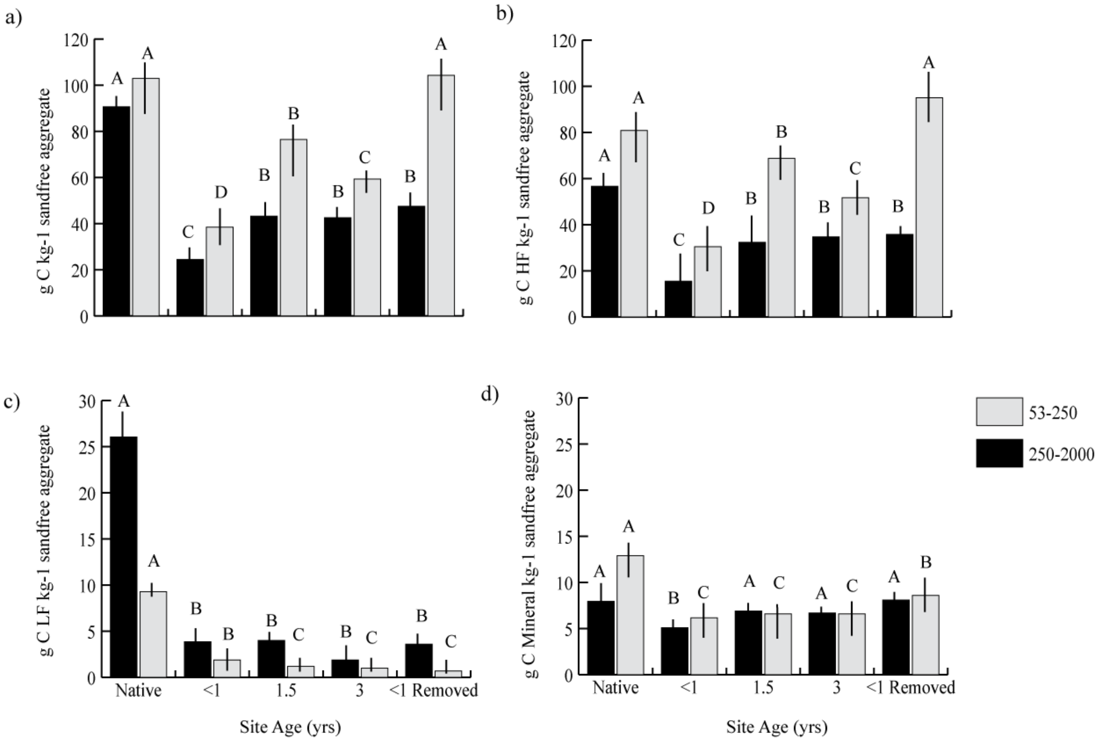

Figure. 9. Carbon concentrations for stockpiled (<1, 1.5, 3, and <1 Removed) and native sites at Belle Ayr Mine, Gillette, WY. Aggregate sizes include a) macro- and microaggregates b) aggregate associated heavy fraction (HF), c) aggregate associated light fraction (LF), and d) aggregate associated mineral fraction (Mineral) Significance shown among site ages at $P<0.05$. Bars represent standard deviation, n=12.

Heavy fraction C (containing both sand and iPOM) for both macro- and microaggregates represented almost 80% of total aggregate associated C on a weight basis (Fig. 9b). As a result, trends similar to those observed in this fraction were also found for aggregate associated C. Light fraction C was almost seven times greater for macroaggregates in the native (26.06 g C g^{-1} sandfree aggregate) compared to stockpiled soils (Fig. 9c). An interesting decrease in macroaggregate LF C, the most available C for microbial decomposition, was observed following 3 yrs of storage. Macroaggregate LF C decreased from 4.00 g C g^{-1} sandfree aggregate in the 1.5 yr sampling to 1.90 g C g^{-1} sandfree aggregate in the 3rd yr of sampling. During the 3rd yr of sampling, the stockpile was extremely moist which could have stimulated microbial activity contributing to macroaggregate formation and potentially increased decomposition of available OM (LF) in the macroaggregate fraction. In agricultural soils, losses in HF and LF C were also most apparent (Besnard et al., 1996; Six et al., 1998). Increases in aggregate C through time have been attributed to contributions from LF C (Tan et al., 2007). This was not observed in stockpiled soils because contributions to macroaggregate C by LF C were 10-15% in stockpiled soils and 30% in the native soils.

There was an interesting decrease in macroaggregate Mineral C (5.10 g C g^{-1} sandfree aggregate) after topsoil stripping. This was followed by a significant increase to pre-disturbance levels (7.95 g C g^{-1} sandfree aggregate) after 1.5 yrs. Microaggregate Mineral C was also reduced through topsoil stripping to nearly half that observed in the native soil; however, concentrations did not reach native C concentrations within 3 yrs. A decline in mineral fraction C associated with disturbance is not commonly observed with other types of disturbance (tillage, land use change, woody encroachment) and the relatively rapid increase in C concentrations in this fraction following a mining disturbance is also uncommon relative to other types of disturbance (Six et al., 2000, 2001; Liao et al., 2006; Schwendenmann and Pendall, 2006). Decreases in this fraction indicate the magnitude of mining disturbances on soil properties; however, the relatively quick recovery in this fraction towards a native soil condition shows the resilience of these soil systems.

Organic Matter Dynamics

Stable C isotopes proved to be a useful tool in understanding OM incorporation into soil aggregates in previous research (Jastrow et al., 1996; Six et al., 2000; Lobe et al., 2005; Schwendenmann and Pendall, 2006; Wick, 2007). This isotopic technique has not been utilized in short term field experiments (<5 yrs). The unique shift in plant communities with revegetation of stockpiles from C_3/C_4 mix to C_3 allowed the use of this method to gain an understanding of where and if OM was being incorporated in the soil matrix. Previous work by Wick (2007) has indicated >50% of aggregate C was recently input C using this method on a chronosequence representing 26 yrs of reclamation. In this study, whole soil $\delta^{13}C$ values decreased with stockpile age from -20.5 ‰ in the native to -21.8 ‰ after 3 yrs of storage (Fig. 10). Macro- and microaggregates showed similar decreases in a 3 yr time period from -21.7 ‰ to -22.2 ‰ and -19.6 ‰ to -21.7 ‰, respectively (Fig. 10). These decreases represent 55% new C in whole soil samples, 42% in macroaggregates and 64% in microaggregates after 3 yrs of storage (Fig. 4). Other studies addressing land use change disturbances found 21-38% of total C in whole soils to be new C after 90 years in cultivated systems (Lobe et al., 2005) and 30-60% of new C associated with soil aggregation after more than 90 years in tropical soils (Schwendenmann and Pendall, 2006). Jastrow et al. (1996) found incorporation of new OM from C_3 plant residue in cropland soils converted to tall grass prairie was more prevalent in larger aggregate size classes. Andreux et al. (1990) and Lobe et al. (2005) observed trends similar to those reported by Jastrow et al. (1996), with most of the younger C_3 plant material incorporated into coarse size fractions (macroaggregates) compared to microaggregate fractions. Though OM held within soil aggregates is generally considered the most stable (Cambardella and Elliott, 1994; Golchin et al., 1995), this material may not be as stable as originally hypothesized (Schwendenmann and Pendall, 2006).

Following the second topsoil movement (<1 Removed site), all δ^{13}C signatures increased slightly (Fig. 10). New C decreased from 55 to 17% in whole soils, 42 to 14% in macroaggregates and 64 to 61% in microaggregates (Fig. 11), possibly indicating decomposition of more recent plant material from stockpile vegetation rather than more humified OM from the native site. It is interesting that there was a small decrease in microaggregate new C following the second movement. This could be the result of more resistant or stable OM in this aggregate size class.

No trends were observed in stable C isotopes in the aggregate fractions (LF, HF, Mineral) over a 3 yr time period (Fig. 12). This was not surprising as most of the changes were expected to be observed in the actual aggregate size classes (Jastrow, 1996; Lobe et al., 2005; Schwendenmann and Pendall, 2006). Changes in δ^{13}C signatures of aggregate fractions were observed by Wick (2007) in a chronosequence spanning 26 yrs, with large incorporations of new C into heavy and Mineral fractions. Three years of storage in a stockpile was apparently not long enough to detect incorporation of new OM into aggregate fractions in this study.

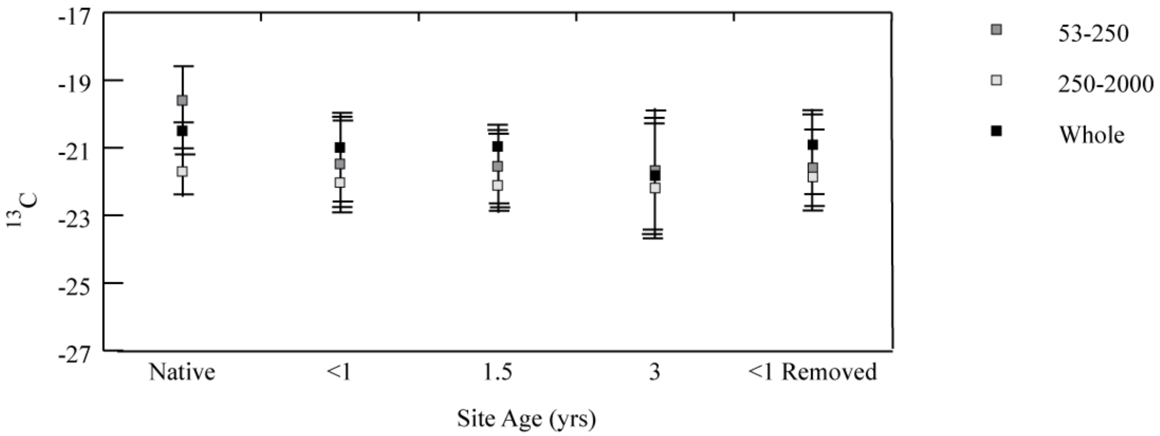

Figure. 10. δ^{13}C signatures of whole and aggregate associated organic matter for stockpiled (<1, 1.5, 3 and <1 Removed) and native soils at Belle Ayr Mine, Gillette, WY. Size fractions include microaggregates (53-250 μm), macroaggregates (250-2000 μm), and whole (total soil). Bars represent standard deviation, n=12.

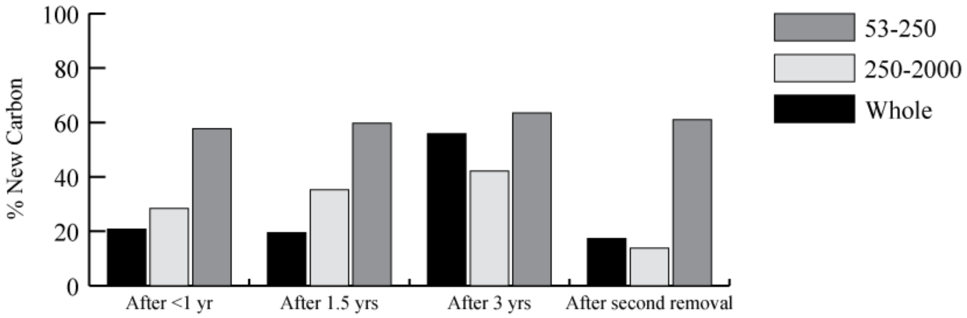

Figure. 11. Percent new carbon for whole soil and aggregate fractions for stockpiled (<1, 1.5, 3 and <1 Removed) and native soils at Belle Ayr Mine, Gillette, WY. Size fractions include microaggregates (53-250 μm), macroaggregates (250-2000 μm), and whole (total soil).

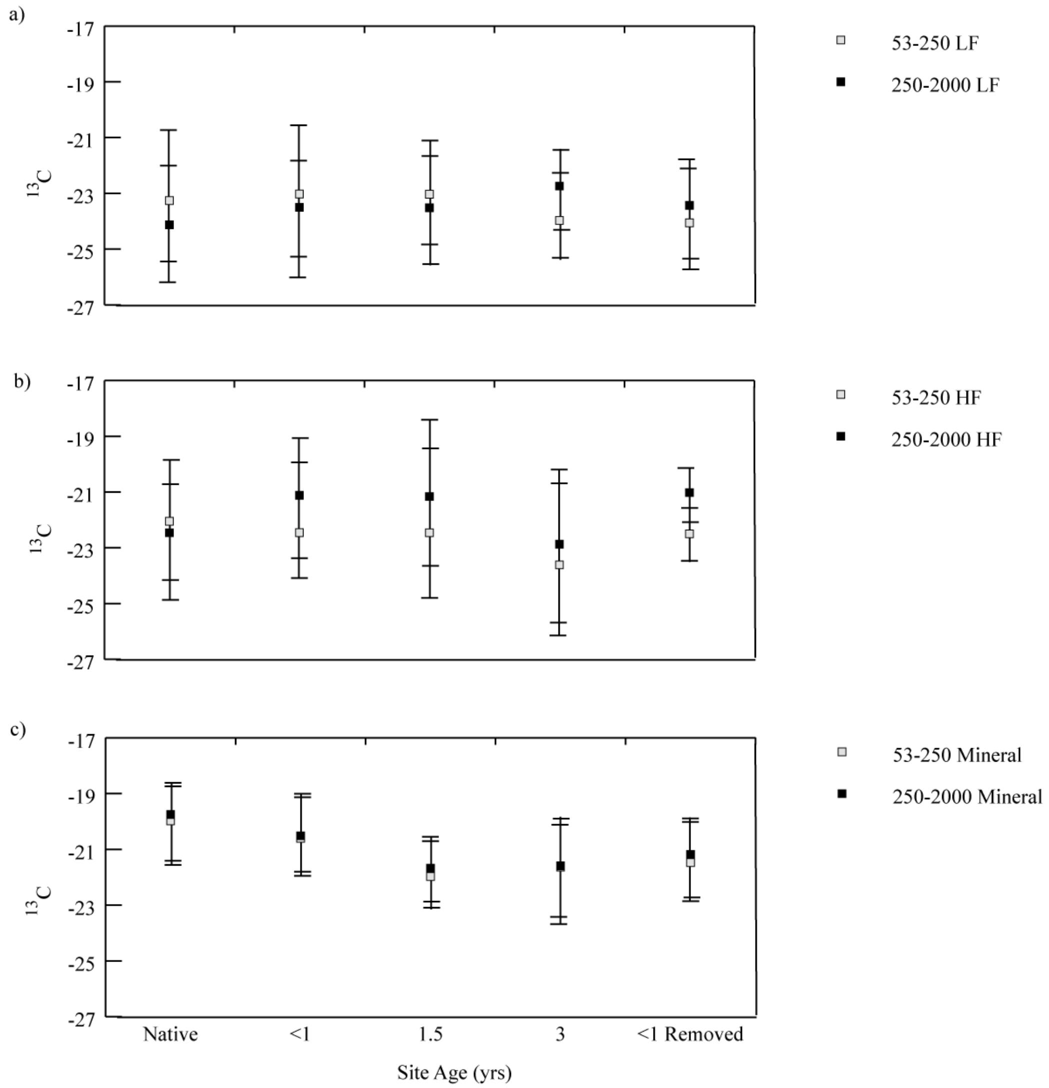

Figure. 12. $\delta^{13}C$ signatures of aggregate associated a) light fraction (LF), b) heavy fraction (HF) particulate organic matter, and c) Mineral fraction for a C_3 grass chronosequence at Belle Ayr Mine, Gillette, WY. Aggregate size fractions include microaggregates (53-250 μm) and macroaggregates (250-2000 μm). Bars represent standard deviation, n=5 for reclaimed sites, n=15 for native site through utilization of 5 samples per depth to account for mixing of the soil profile.

Conclusions

Storing topsoil in stockpiles has important implications for reclamation success. Stockpiling has previously been shown to negatively impact favorable soil properties (such as, but not limited to, microbial biomass, bulk density, water holding capacity, and viable seed populations) mainly at depth; however, stockpiling has been shown in this study to increase stockpile surface aggregation and aggregate associated organic C concentrations after 3 yrs of storage. Despite the decrease in large aggregation of stockpile surface soils following the second movement of stored topsoil, C concentrations remained unchanged in surface soils. Pockets of C created in the surface of stockpiled soils could further increase the heterogeneity of reclaimed soils and serve as "hot spots" for biological activity, though it is important to remember that surface soils are considered to be a small portion of the stockpiled soils. Further research on aggregate and OM dynamics could be conducted on topsoil stored for longer periods of time with greater depth measurements and tracked to reclaimed areas. Additionally, this study has shown that recovery of aggregate and C pools following disturbance may begin in a short period of time.

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