REDEVELOPMENT OF SOIL CARBON POOLS ON RECLAIMED SURFACE MINE LANDS[1]

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Abstract. Soils play a crucial role in the global carbon cycle; they represent a carbon reservoir larger than the atmosphere, they are the site of a number of key carbon transformations (e.g. mineralization and humification), and contain a number of unique carbon pools (e.g. plant litter, humic substances, dissolved organic matter, etc.). Land disturbance associated with surface coal mining results in complete disruption of the soil system, loss of a significant portion of soil carbon content, and disturbance of many of the organisms that play critical roles in the carbon cycle (primary producers and decomposers). One of the challenges of surface mine reclamation is reconstruction of a soil system which functions properly in the impacted ecosystem. The objective of this paper is to report our research findings on the recovery of soil carbon pools in reclaimed surface mined lands. Data from our studies indicate 2 mechanisms are important in the rapid accumulation of C from plant litter into soil: physical protection by soil aggregates and biochemical protection of high lignin content. Examination of chronosequences of reclaimed soils indicates plant litter is rapidly incorporated into soil aggregate structure in most of these soils. Lignin content of reclaimed soils we analyzed were higher than that of nearby undisturbed soils, indicating the recalcitrant nature of soil C in reclaimed soils and/or possibly the slow recovery of lignin degrading organisms, primarily fungi. Assays of potentially mineralizable C indicate concentrations of labile C in reclaimed soils reach amounts similar to those in undisturbed soil within 5 or 6 years after revegetation.

Additional Key Words: Ecosystem Recovery, Soil Organic Matter, Mineralizable Carbon, chronosequences. Soil Aggregates

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Introduction

Soils contain vast amounts of carbon in the form of soil organic matter (SOM), which includes plant, microbial, and animal residues in various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by soil microorganisms (Sylvia et al., 2005). Carbon content of SOM generally ranges from 40-58% (Brady and Weil, 2000). Soil organic matter exerts a dominant positive influence on many soil physical, chemical and biological properties and is a critical factor determining soil quality (Stevenson and Cole, 1999).

One of the most commonly observed impacts of soil disturbance and degradation is loss of SOM and associated soil organic carbon (SOC) (Harris et al., 1996). The moderate disturbance of soil caused by agricultural tillage has resulted in significant losses of SOC in cultivated soils throughout the world. Loss of SOC or SOM has been commonly reported for soils disturbed by activities associated with surface mining (Visser et al., 1984; Harris et al., 1993; Stahl et al., 2002)

Reclamation of disturbed and degraded soils generally leads to redevelopment of SOM in the form of plant, microbial and animal residues (Stahl et al., 2003), which greatly contributes to improvement of soil fertility as well as soil structure, water holding capacity, biotic activity, and plant growth (Harris et al., 1996; Lal et al., 1998). Reestablishment of soil carbon pools in disturbed soil also sequesters carbon from the atmosphere where it is contributing to global climate change.

The objective of this paper is to report our research findings on the recovery of soil carbon pools in reclaimed surface mined lands and describe important mechanisms by which SOC is protected from decomposition in reclaimed soils; physical protection by soil aggregates and biochemical protection of high lignin content.

Methods

Two chronosequences of reclaimed surface coal mine sites were used to examine the recovery of reclaimed soils over time. Chronosequences are useful in reclamation research for observation of site recovery over time, ecosystem change and evaluation of specific reclamation practices or techniques.

Reclaimed sites were sampled on two surface coal mines located in the Powder River Basin of northeastern Wyoming, USA. At the Dave Johnston Mine (N 43°03’/W 105°82’) in Converse

County, a chronosequence of four reclaimed shrub sites (<1, 5, 10, 16 years old) and an undisturbed site were sampled. Vegetation at sites in this chronosequence were dominated by Wyoming big sagebrush [*Artemisia tridentate* var. Wyomingensis (Beetle and Young) Welsh], prairie sagewort [*Artemisia frigida* Willd.], and Idaho fescue [*Festuca idahoensis* Elmer.]. Average annual precipitation at this mine is 305 mm, mean air temperature 8.7°C and elevation is 1646 m (Western Region Climate Center 2006). Prior to mining, soils were classified as fine-loamy, mixed, mesic Ustic Haplargids (Westerman and Prink 2004; Munn and Arneson 1999).

At the Belle Ayr Mine (N 44°10'/W 105°27') in Campbell County, a chronosequence of a topsoil stockpile (<1 year) to represent recent reclamation and two reclaimed sites (14 and 26 years old) at which vegetation was dominated by cool season grasses and an undisturbed site were sampled. The cool season grasses seeded on the reclaimed sites included western wheatgrass [*Pascopyrum smithii* (Rydb.) A. Löve], green needlegrass [*Nassella viridula* (Trin.) Barkw.], and needle and thread grass [*Hesperostipa comata* (Trin. & Rupr.) Barkw]. Average annual precipitation at this mine is 390 mm, mean air temperature 6.7°C and elevation is 1375 m (Western Region Climate Center 2006). Prior to mining, soils were also classified as fine-loamy, mixed, mesic Ustic Haplargids (Westerman and Prink 2004; Munn and Arneson 1999). Each site sampled had similar soil type, topography (<1 percent slope) and was approximately 0.5 ha in size. Direct haul topsoil replacement (except at stockpile site) and similar seed mixes were used at each site.

Soil samples were collected from both chronosequences in May 2005 when soils were moist and plants were beginning to develop. At each different aged site, three 45 meter long randomly placed transects were laid out and soil was sampled at four evenly spaced points along each transect. Samples collected for SOM characterization were composited along each transect. The top 5 cm of soil was collected with a hand trowel and the 5-15 and 15-30 cm depths with a 2.5 cm diameter step probe. Five volumetric soil samples were collected for determination of bulk density (BD) and root biomass at each site using a double-cylinder, hammer driven core sampler (Grossman and Reinsch 2002).

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) using the hydrometer method (Gee and Or, 2002). Electrical conductivity (EC) and pH were determined on all samples with 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 carbon (C) and nitrogen (N) values were obtained on all samples which were finely ground (<53 μm) prior to analysis by dry combustion with a Carlo Erba NC 2100 Analyzer (Lakewood, NJ). Inorganic C (IC) content of soil was also determined on finely ground samples with the modified pressure calcimeter method (Sherrod et al., 2002). Soil organic C (SOC) content was determined by subtracting IC from total C.

Soil Lignin

Soil lignin was determined on subsamples from all three depths that were finely ground to pass through a 200 µm sieve. Alkaline cupric oxide (CuO) oxidation was conducted in a pressure bomb using a modified method developed by Kögel and Böchter (1985) and Kögel-Knabner *et al*. (1991). Lignin oxidation products were quantified using a Shimadzu HPLC system consisting of LC-10AS solvent delivery unit, SIL-9A auto injector (20 µl of solution) unit and a SPD-M6A UV detector set at 280 nm. Separation was achieved using a 25 cm x 4 mm i.d. column filled with Nucleosil C18 (3µm) stationary phase in combination with a nucleosil C18 (5 µm) cartridge placed immediately before the column. Gradient elution was carried out at a flow rate of 0.8 ml min^{-1}. Eluent A was 50 mM phosphate buffer (pH 2.1) containing 10% acetonitrile and eluent B contained acetonitrile with a 30% phosphate buffer (pH 2.1). The gradient started with 97% eluent A and 3% eluent B, and was terminated after 60 min when eluents A and B were 90% and 10%, respectively. The column was purged with pure eluent B for 10 min and then re-equilibrated with 97% eluent A and 3% B for 10 min. Lignin contents of soils from different sites were obtained by using lignin concentrations, soil bulk density, and TOC concentrations for respective sites.

Aggregates and Aggregate Fractions

Aggregate size classes (250-2000, and 53-250 μm) were isolated from whole soil samples using a wet sieving technique described by Six et al. (1998). Aggregate samples were then analyzed for particulate organic matter (POM) fractions according to methods described by Six et al. (1998) on the 0-5 cm depth. 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, Free 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 (iPOM, sand, silt and clay) was rinsed twice with deionized water, flocculated with 5 drops of 0.25 M $CaCl_2$ + 0.25 M $MgCl_2$ solution and centrifuged 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+Sand) and material washed through the sieve (Silt+Clay) were dried at 55˚C overnight. Flow chart of fractions presented in Fig. 1.

Aggregate Carbon and Nitrogen

All samples (macro- and microaggregates, POM fractions, and roots) were analyzed for total C using dry combustion on an Elementar Variomacro Analyzer (Hanau, Germany). Aggregate

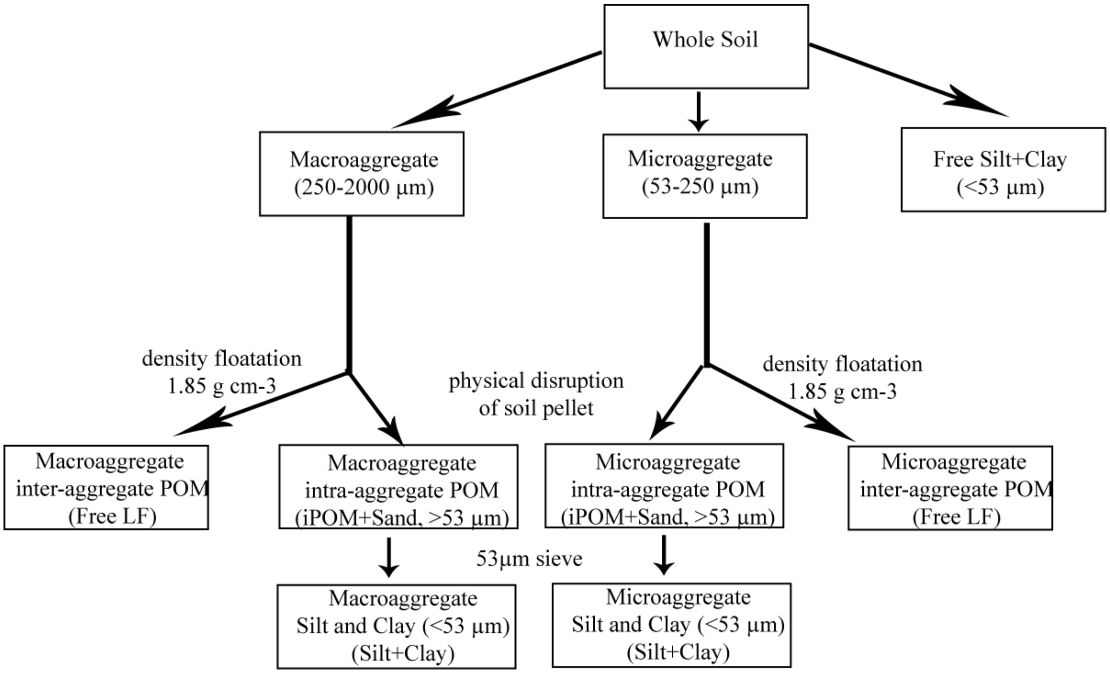

Figure 1. Flow chart for aggregate size distributions and separation of aggregate fractions.

and whole soil samples from the Belle Ayr Mine chronosequence were analyzed for ^{13}C with a Thermo Finnigan Delta Plus XP Continuous Flow Stable Isotope Ratio Mass Spectrometer (Bremen, Germany). The unique shift in plant communities allowed the use of stable carbon isotopes to identify shifts in SOM pools. Weighted averages for the native soils were calculated based on C concentration and isotopic signature for each depth to get a better representation of the soil mixing that occurs as a result of stripping and respreading during reclamation. Inorganic C was not detected in the whole soil or aggregate samples so total C was considered organic C. Comparisons of C and N concentrations across sites are not valid unless corrected for sand (Elliott et al., 1991). The following formulas were used to calculate the sand free C content (Equation 1) for each size class (Denef et al., 2001):

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

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

$$\%C_{\text{new}} = [(\delta^{13}C_{\text{reclaimed}} - \delta^{13}C_{\text{native}})/(\delta^{13}C_{\text{roots}} - \delta^{13}C_{\text{native}})] \times 100 \tag{2}$$

Where $\delta^{13}C_{\text{reclaimed}} = \delta^{13}C$ of SOM in bulk and aggregate fraction in reclaimed soils, $\delta^{13}C_{\text{native}}$ = mean $\delta^{13}C$ of SOM in bulk and aggregate fraction in native soils, and $\delta^{13}C_{\text{roots}} = \delta^{13}C$ of fine root material for reclaimed sites.

Mean residence time (MRT) for macroaggregate associated C and N derived from native vegetation (pre-mining) was calculated using a first-order decay model (Six and Jastrow, 2002) (Equation 3).

$$\text{MRT} = -[t / \ln(C_t/C_o)] \tag{3}$$

Where t is the time since reclamation initiation (26 y), C_t is the concentration (g C or N kg^{-1} sand free macroaggregate) from the <1 y old site, and C_o is the concentration present in the 26 y old site.

Mineralizable Carbon Assay

The amount of carbon potentially available for microbial mineralization was quantified using an incubation assay on the 0-5 cm depth (Zibilske, 1994; Franzluebbers et al., 2000). For this assay, 22 g of field moist soil was placed in 120-mL plastic specimen cups. A calculated amount of DI water was added to the soil to increase water content to 0.23 g H_2O g^{-1} oven dry soil which was an equivalent of the amount of soil water present at field capacity. Plastic cups containing soil and added water were placed in 1-L Mason jars. In order to assure sufficient air humidity in jar headspace, 2 mL of D.I. H_2O were added to the bottom of each jar prior to the specimen cup placement. Jars were sealed with lids containing rubber septa and placed in a dark incubator at 23°C for 21 days.

Carbon mineralization rates were measured by drawing a sample of headspace gas at 3 hours, 1, 2, 3, 7, 14, and 21 days of incubation. Gas samples were analyzed for CO_2 concentration using a Licor infra-red gas analyzer (IRGA). Following each sampling time, all jars were opened, the remains of gas accumulated in jar headspace evacuated and sealed again. At the end of the 21-d incubation, soil was removed from jars and two 10 g subsamples were immediately extracted with 0.5 M K_2SO_4 to determine remaining extractable carbon content. This last fraction is thought to represent non-humic and humic substances once adsorbed to mineral surfaces.

The cumulative amount of CO_2 produced from moist, homogenized soils during the entire 21-d incubation represents the potentially mineralizable C pool, and expressed as mg C kg^{-1} oven-dry soil. The value is considered to be an index of the quantity of labile C present in the soil at the time of sampling (Zibilske, 1994).

Results

General Soil Properties

Reclaimed soils present on the shrub chronosequence at the Dave Johnston Mine were primarily of sandy loam texture (Table 1). A decrease in bulk density was found with reclamation age. Soil organic C and N increased with reclamation age and soil depth. Soil pH was lower in the undisturbed soils than the reclaimed soils and EC generally decreased with reclamation age.

Reclaimed soils present on the cool season grass chronosequence at the Belle Ayr Mine were primarily of sandy clay loam texture (Table 2). Bulk density was lower in the undisturbed site compared to the reclaimed sites. Soil organic C and N increased with reclamation age and soil depth. Soil pH was lower in the native site than the reclaimed sites and EC generally decreased with reclamation age.

Table 1. General soil properties of a reclaimed chronosequence at the Dave Johnston Mine, Glenrock, WY. Data from Wick, 2007.

Site Age	Depth	Sand	Silt	Clay	Bulk Density	SOC[1]	N	EC	pH
(yrs)	(cm)	g 100 g-1 soil			g cm^{-3}	Mg ha^{-1}	Mg ha^{-1}	μS cm^{-1}	
<1	0-5	65	16	19	1.49 ± 0.06	4.62 ± 0.02	0.413± 0.00	483.20± 35.66	7.6± 0.1
	5-15	68	13	19	1.44 ± 0.08	8.35 ± 0.04	6.48± 0.00	472.20± 41.02	7.5± 0.1
5	0-5	64	22	14	1.55± 0.04	5.36± 0.04	4.37 ± 0.00	269.50± 14.31	7.9± 0.1
	5-15	66	19	15	1.51± 0.06	9.09± 0.02	7.75 ± 0.00	200.70± 3.63	8.0± 0.1
10	0-5	67	20	13	1.59± 0.06	6.28± 0.05	5.83 ± 0.03	224.60± 14.49	7.4± 0.1
	5-15	66	22	12	1.49± 0.04	8.34± 0.03	7.20± 0.00	205.00± 4.52	8.1± 0.1
16	0-5	61	27	12	1.38± 0.04	8.35± 0.14	8.51± 0.01	213.30± 13.49	7.2± 0.1
	5-15	62	25	13	1.45± 0.06	9.28± 0.06	9.47± 0.00	200.90± 13.77	7.7± 0.1
Undisturbed	0-5	55	29	16	1.26± 0.14	12.85± 0.28	21.90 ± 0.01	253.70± 26.76	6.2± 0.1
	5-15	58	30	12	1.39± 0.05	15.15± 0.24	11.20± 0.01	96.24± 8.93	6.6± 0.1

TOC and IC at the DJ chronosequence

Soil IC concentrations ranged from 0.44 g kg^{-1} in the 5-15 cm depth of undisturbed soil to 0.99 g kg^{-1} in the 15-30 cm depth of 16 yr old site (Table 3). Soil IC concentrations did not vary significantly among sites or among different depths. The TOC concentrations in DJ chronosequence reclaimed soils ranged from 4.13 to 8.29 g kg^{-1} soil (Table 3). Total OC concentrations in DJ reclaimed soils were significantly (p=0.002) lower than that of undisturbed soils at all depths, except the 10 yr old reclaimed site at 0-5 and 5-15 cm and 5 yr old reclaimed site at 15-30 cm.

Total OC content did not change with time in DJ reclaimed soils at any depth. There was no clear trend for SOC content, although values tend to stabilize at 15-30 cm depth after 10 yrs. Undisturbed soil and 5 yr old reclaimed site SOC contents at 15-30 cm depth were significantly (p=0.009) greater than that of 0-5 cm depth soils. In the 10 yr old reclaimed site, 5-15 cm depth soils contained significantly greater SOC content than 0-5 cm depth soils and TOC content did not vary among different depths in 16 yr old reclaimed site. Total SOC content of reclaimed site soils in the top 30 cm depth did not vary significantly with time and undisturbed TOC contents were significantly (p<0.001) greater than reclaimed soils at 0-30 cm (Table 3).

Table 2. General soil properties of a reclaimed chronosequence of sites at the Belle Ayr Mine, Gillette, WY. Data from Wick, 2007.

Site Age	Depth	Sand	Silt	Clay	Bulk Density	SOC[1]	N	EC	pH
(yrs)	(cm)	g 100 g^{-1} soil			g cm^{-3}	Mg ha^{-1}	Mg ha^{-1}	μS cm^{-1}	
<1	0-5	39	34	27	1.26± 0.07	5.54± 0.06	5.90± 0.00	516.90± 17.06	7.8± 0.1
	5-15	36	35	29	1.35± 0.06	9.85± 0.05	11.20 ± 0.00	555.90± 35.48	7.6± 0.1
14	0-5	38	28	34	1.20± 0.06	19.68± 0.24	6.85± 0.01	371.30± 12.81	7.6± 0.1
	5-15	38	26	36	1.33± 0.04	14.23± 0.08	18.20± 0.00	368.80± 11.11	8.1± 0.1
26	0-5	38	33	29	1.21± 0.04	13.43 ± 0.33	7.94± 0.02	413.90± 36.07	7.0± 0.1
	5-15	37	30	33	1.38± 0.02	13.39± 0.06	9.61± 0.00	305.00± 19.82	7.8± 0.1
Undisturbed	0-5	53	26	21	1.34± 0.04	9.92± 0.10	8.11± 0.01	156.50± 17.54	6.6 ±0.1
	5-15	54	21	26	1.44± 0.04	14.26± 0.11	12.00± 0.01	186.10± 17.04	6.9± 0.1

Carbon and Inorganic Carbon at the BA Chronosequence

Soil IC concentration ranged from 1.63 to 8.47 g kg^{-1} in reclaimed mine soils and 0.96 to 1.06 g kg^{-1} in undisturbed soils (Table 4). Soil IC concentrations were significantly (p<0.001) greater in 14 yr old reclaimed site than other sites at 0-5 and 5-15 cm depths; IC concentrations at 15-30 cm in the 14 yr old reclamation were significantly greater than that of the 26 yr old reclamation and undisturbed soil. Soil IC concentrations in the 5-15 cm depth were significantly greater than 0-5 and 15-30 cm depths. Inorganic C concentrations in the 15-30 cm depth were significantly greater than other two depths in the 26 yr old reclaimed site and IC concentration at 5-15 cm in the 14 yr old reclaimed site was significantly greater than other two depths for that site.

Table 3: Concentrations of soil inorganic carbon, total organic carbon, total nitrogen, and C:N ratios in Dave Johnston chronosequence soils.

Depth (cm)	Undisturbed	5 Years	10 Years	16 Years
		Soil Inorganic C (g kg^{-1})		
0-5	0.60^{aA}	0.87^{aA}	0.87^{aA}	0.65^{aA}
5-15	0.44^{aA}	0.90^{aA}	0.70^{aA}	0.92^{aA}
15-30	0.58^{aA}	0.67^{aA}	0.91^{aA}	0.99^{aA}
		Total Organic C (g kg^{-1})		
0-5	18.69^{aA}	6.67^{bA}	8.29^{abA}	7.43^{bA}
5-15	12.40^{aAB}	6.25^{bA}	8.30^{abA}	4.98^{bA}
15-30	9.41^{aB}	6.77^{abA}	4.50^{bA}	4.13^{bA}
		C:N		
0-5	14.61^{aA}	11.53^{abA}	9.86^{bA}	10.45^{abA}
5-15	12.00^{aAB}	11.94^{aA}	11.36^{abA}	9.33^{bA}
15-30	9.32^{bB}	14.03^{aA}	9.67^{bA}	8.61^{bA}

Lower case letters represent significant differences among reclaimed sites and undisturbed site samples within a mine at the same depth ($p \leq 0.05$). Capital case letters represent significant differences among different depths within the site ($p \leq 0.05$).

Table 4: Concentrations of total organic carbon, total nitrogen, and C:N ratios in Belle Ayr chronosequence soils.

Depth (cm)	Undisturbed	<1 Year	14 Years	26 Years
		Soil Inorganic C (g kg^{-1})		
0-5	0.86^{bA}	2.80^{bA}	5.77^{aB}	2.36^{bB}
5-15	0.74^{cA}	3.94^{bA}	8.47^{aA}	1.82^{cB}
15-30	1.06^{cA}	2.54^{abA}	6.27^{aB}	4.46^{bA}
		Total Organic C (g kg^{-1})		
0-5	26.51^{aA}	6.16^{bA}	20.22^{abA}	14.20^{bA}
5-15	10.99^{aAB}	5.46^{bA}	5.77^{bB}	8.95^{abA}
15-30	8.02^{aB}	5.37^{aA}	5.68^{aB}	10.01^{aA}
		C:N		
0-5	10.81^{bA}	9.64^{bA}	14.26^{aA}	11.31^{abA}
5-15	9.23^{aA}	10.92^{aA}	10.19^{aA}	9.82^{aA}
15-30	11.46^{aA}	9.03^{aA}	10.58^{aA}	10.50^{aA}

Lower case letters represent significant differences among reclaimed sites and undisturbed site samples within a mine at the same depth ($p \leq 0.05$). Capital case letters represent significant differences among different depths within the site ($p \leq 0.05$).

Total OC concentrations were generally greater in 0-5 cm of reclaimed and undisturbed soils (Table 4). In reclaimed soils, maximum C concentrations were observed in the 14 yr old reclaimed 0-5 cm depth, although differences were not statistically significant. The C concentration of the undisturbed soil was significantly ($p<0.001$) greater than that of reclaimed soils at 0-5 and 5-15 cm, except the 14 yr old reclamation 0-5 cm and 26 yr old 5-15 cm depth. No significant differences were observed among undisturbed and reclaimed at 15-30 cm. Total OC concentrations of the <1 yr topsoil site did not significantly differ from the 16 yr old stockpile at any depth.

Total SOC content in 0-30 cm depth of BA reclaimed sites increased significantly ($p=0.050$) from 24.1 Mg ha^{-1} in <1 yr old to 38.4 Mg ha^{-1} in 26 yr old reclaimed mine soil (Table 4). Total SOC content of undisturbed soil was significantly ($p=0.020$) greater than that of reclaimed sites in the 0-30 cm.

Lignin

Concentrations of vanillyl, syringyl, and cinnamyl groups, Ac/Al$_V$, Ac/Al$_S$, and C:V ratios of soils at two mines are provided in Tables 4 and 5, respectively. Vanillyl and Syringyl groups constituted most of the lignin in these soils.

Dave Johnson Mine Chronosequence**.** Vanillyl concentrations in the 10 yr old reclamation were significantly ($p<0.001$) greater than other sites at the 0-5 cm depth; the 16 yr old reclaimed soils contained greater vanillyl concentration than undisturbed site at 5-15 cm, and contained significantly greater vanillyl concentration than undisturbed and DJ5 yr old reclaimed site at 15-30 cm (Table 5). Syringyl concentrations were significantly ($p<0.001$) different only at 0-5 cm depth with the 10 yr old reclamation having greater concentrations than undisturbed soil (Table 5). Cinnamyl concentration did not vary among sites or depths. The Ac:Al$_V$ values of undisturbed soils were significantly ($p=0.001$) greater than reclaimed mine soils at 0-5 and 5-15 cm depths. At the 15-30 cm depth of the 16 yr old reclaimed site, Ac:Al$_V$ ratios were significantly greater than that of 5 and 10 yr old reclamation. The Ac:Al$_V$ ratio differences were significant among depths only in undisturbed with 0-5 cm having higher values. The Ac:Al$_S$ ratios in undisturbed soils were significantly greater than their respective reclaimed mine sites. Among reclaimed soils, Ac:Al$_S$ ratios increased significantly ($p<0.001$) with time only at the 5-15 cm depth. The C:V ratios were significantly ($p=0.007$) greater at the 5 yr old site compared to other DJ sites at the 0-5 cm soil depth (Table 5).

Table 5: Concentrations of VSC (Vanillyl, Syringyl, Cinnamyl), Ac/Al$_V$, Ac/Al$_S$, and C:V ratios in Dave Johnston chronosequence soils.

Depth (cm)	Undisturbed	5 Years	10 Years	16 Years
Vanillyl (mg kg^{-1} TOC)				
0-5	2.39^{bA}	2.83^{bA}	6.72^{aA}	4.57^{abA}
5-15	1.05^{bA}	3.55^{abA}	3.35^{abB}	6.05^{aA}
15-30	0.77^{bA}	1.43^{bA}	4.91^{abAB}	5.16^{aA}
Syringyl (mg kg^{-1} TOC)				
0-5	1.25^{bA}	2.57^{abA}	5.00^{aA}	3.30^{abA}
5-15	1.38^{aA}	2.82^{aA}	2.30^{aA}	2.99^{aA}
15-30	0.76^{aA}	1.50^{aA}	2.90^{aA}	2.65^{aA}
Cinnamyl (mg kg^{-1} TOC)				
0-5	0.12^{aA}	0.55^{aA}	0.52^{aA}	0.23^{aA}
5-15	0.09^{aA}	0.30^{aA}	0.31^{aA}	0.12^{aA}
15-30	0.04^{aA}	0.22^{aA}	0.27^{aA}	0.10^{aA}
Ac/Al$_V$				
0-5	0.624^{aA}	0.154^{dA}	0.323^{cA}	0.436^{bA}
5-15	0.529^{aB}	0.087^{dA}	0.282^{cA}	0.457^{bA}
15-30	0.398^{abC}	0.085^{cA}	0.334^{bA}	0.415^{aA}
Ac/Al$_S$				
0-5	0.717^{aA}	0.277^{bA}	0.368^{abA}	0.382^{abA}
5-15	0.691^{aB}	0.241^{cA}	0.314^{bAB}	0.333^{bA}
15-30	0.466^{aC}	0.249^{bA}	0.255^{bB}	0.307^{bA}
C:V				
0-5	0.043^{bA}	0.239^{aA}	0.054^{bA}	0.049^{bA}
5-15	0.089^{aA}	0.147^{aA}	0.092^{aA}	0.019^{aA}
15-30	0.057^{aA}	0.148^{aA}	0.050^{aA}	0.018^{aA}

Lower case letters represent significant differences among reclaimed sites and undisturbed site samples within a mine at the same depth ($p \leq 0.05$). Capital case letters represent significant differences among different depths within the site ($p \leq 0.05$).

Reclaimed soils of DJ shrub chronosequence also showed an increase in lignin content of TOC with time in the 0-30 cm depth soils but this increase was not statistically significant. The lignin content was significantly (p=0.008) greater in reclaimed sites than the undisturbed site at 5-15 and 15-30 cm depth soils. The mean lignin content increased from 5.95 to 8.10, 6.67 to 9.16, 3.15 to 7.91g lignin kg^{-1} TOC at 0-5, 5-15, and 15-30 cm depths over 11 yr. Range for lignin content was much higher (2.4 to 26.5 g lignin kg^{-1} TOC) for reclaimed sites compared to their respective undisturbed sites (1.1 to 6.3 g lignin kg^{-1} TOC). Among different depths, only the 10 yr old reclaimed site contained significantly greater lignin contents at 0-5 cm than at 5-15 cm.

Belle Ayr Mine Chronosequence. Total OC lignin content in the 26 yr old reclaimed soils was significantly (*p*<0.001) greater than undisturbed soils at 0-5 and 5-15 cm. Lignin content ranged from 5.7 to 17.6 g lignin kg^{-1} TOC in BA mine reclaimed soils for all three depths, which was higher compared to the 2.2 to 8.1 g lignin kg^{-1} TOC range observed in the undisturbed site. In the 14 and 26 yr old reclamation, lignin content increased with depth from 9.1 and 17.6 g lignin kg^{-1} TOC and 9.5 and 16.9 g lignin kg^{-1} TOC in 0-5 and 15-30 cm depth soils, respectively. The proportion of lignin in TOC increased with time in reclaimed soils at 0-30 cm although lignin content differ significantly in the initial 14 yrs. There were significant differences in lignin content among depths with 0-5 cm having greater lignin than 5-15 cm soil depths at <1 yr reclamation and the 15-30 cm having greater lignin contents than the 0-5 cm depth soil in the 26 yr old reclamation.

At 15-30 cm, BA26 had significantly greater vanillyl concentration than other sites. Significant (*p*<0.001) differences in vanillyl concentrations were observed among different depths of undisturbed and <1 yr site (Table 6). Syringyl group concentration varied significantly (*p*=0.002) only at 15-30 cm, with the 26 yr old reclamation having the highest concentration (Table 6). At 15-30 cm, cinnamyl concentration in the 26 yr old reclamation was significantly greater than that in <1 and 14 yr reclamation and undisturbed soils.

The Ac:Al$_V$ ranged from 0.127 to 0.274 in BA reclaimed mine soils, whereas it ranged from 0.196 to 0.490 in undisturbed soils (Table 6). Undisturbed soil had significantly (*p*<0.001) greater Ac:Al$_V$ values than reclaimed soils at 0-5 and 5-15 cm depths. At 15-30 cm, undisturbed, <1 yr and 26 yr reclamation had significantly greater Ac:Al$_V$ than the 14 yr reclamation. The Ac:Al$_S$ ratios in undisturbed soils were significantly greater than their respective reclaimed mine sites and topsoil stockpiles at 0-5 and 5-15 cm. In general the Ac:Al$_S$ ratios increased significantly (*p*<0.001) with age of reclamation. The C:V ratio did not show a definite trend in BA reclaimed sites.

Table 6: Concentrations of VSC (Vanillyl, Syringyl, Cinnamyl), Ac/Al$_V$, Ac/Al$_S$, and C:V ratios in Belle Ayr chronosequence soils.

Depth (cm)	Undisturbed	<1 Year	14 Years	26 Years
Vanillyl (mg kg^{-1} TOC)				
0-5	4.92^{bA}	5.61^{bA}	4.23^{bA}	5.56^{bA}
5-15	1.80^{bAB}	3.28^{bB}	4.86^{abA}	5.81^{abA}
15-30	1.24^{cB}	4.43^{bAB}	4.34^{bA}	7.68^{aA}
Syringyl (mg kg^{-1} TOC)				
0-5	3.01^{aA}	6.34^{aA}	4.28^{aA}	3.08^{aB}
5-15	1.44^{aA}	3.13^{aAB}	3.56^{aA}	4.63^{aB}
15-30	0.84^{cA}	2.06^{bB}	1.39^{bA}	7.75^{aA}
Cinnamyl (mg kg^{-1} TOC)				
0-5	0.24^{bA}	0.80^{abA}	0.43^{bA}	0.68^{abB}
5-15	0.08^{aA}	0.16^{aA}	0.39^{aA}	0.54^{aB}
15-30	0.07^{bA}	0.23^{bA}	0.60^{bA}	1.51^{aA}
Ac/Al$_V$				
0-5	0.490^{aA}	0.226^{bA}	0.249^{bA}	0.240^{bA}
5-15	0.338^{aB}	0.222^{bA}	0.217^{bA}	0.185^{cA}
15-30	0.196^{aC}	0.205^{aA}	0.127^{bB}	0.198^{aA}
Ac/Al$_S$				
0-5	0.568^{aA}	0.273^{cA}	0.321^{cA}	0.422^{bA}
5-15	0.530^{aA}	0.232^{cA}	0.289^{cA}	0.367^{bAB}
15-30	0.342^{aB}	0.253^{bA}	0.284^{aA}	0.332^{aB}
C:V				
0-5	0.048^{aA}	0.156^{aA}	0.101^{aA}	0.121^{aA}
5-15	0.062^{aA}	0.069^{aA}	0.063^{aA}	0.094^{aA}
15-30	0.087^{bA}	0.047^{bA}	0.141^{abA}	0.181^{abA}

Lower case letters represent significant differences among reclaimed sites and undisturbed site samples within a mine at the same depth ($p \leq 0.05$). Capital case letters represent significant differences among different depths within the site ($p \leq 0.05$).

Aggregate Organic Carbon

Macroaggregate C in all fractions reached a concentration similar to that observed in the undisturbed, native soil (15.32 g C kg^{-1} sand free macroaggregate) within 5 yrs of reclamation (21.83 g C kg^{-1} sand free macroaggregate) under shrub dominated plant communities at Dave Johnston Mine (Fig. 2a). No trends were observed for the microaggregate C concentrations for this chronosequence. Macroaggregate C concentrations were consistently higher than that observed in microaggregates and had a much shorter MRT (10 yrs) than microaggregates (115 yrs). Macroaggregate C accumulated at a rate of 0.70 g C kg^{-1} sand free aggregate yr^{-1}, while microaggregate C accumulated at a much slower rate of 0.07 g C kg^{-1} sand free aggregate yr^{-1}. Macroaggregate Free LF C increased significantly between the <1 yr site (0.01 g Free LF C kg-1 sand free macroaggregate) and the 5 yr old reclaimed site (0.04 g Free LF C kg-1 sand free macroaggregate) to a level similar to the undisturbed site (0.05 g Free LF C kg-1 sand free macroaggregate) (Fig. 2b). A majority (>80%) of aggregate C was located in the iPOM+Sand fraction, which followed similar trends to the aggregate fractions (Fig. 2c). Aggregate associated Silt+Clay C did not change with disturbance or with reclamation age (Fig. 2d).

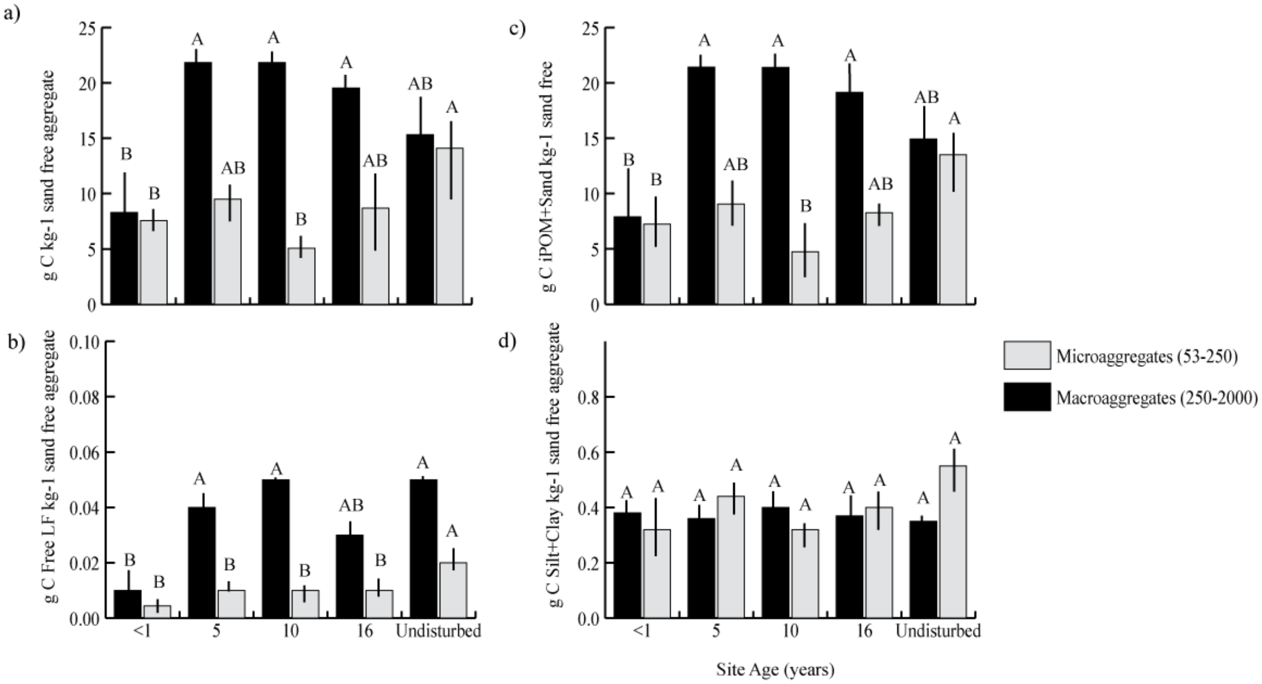

Figure 2. Carbon concentrations in a) macro- and microaggregates b) aggregate associated light fraction (Free LF), c) aggregate associated iPOM+Sand, and d) aggregate Silt+Clay fraction for a shrub chronosequence at Dave Johnston Mine, Glenrock, WY. For a given soil parameter, columns with a different letter above them are significantly different (P<0.05) among site ages. Bars represent one standard deviation, n=3.

Aggregate C concentrations were drastically reduced by topsoil stripping prior to mining at the Belle Ayr Mine. Carbon concentrations were reduced 65-75% for both macro- and microaggregates in the <1 y stockpiled soil compared to the native soil (Fig. 3a). The greatest percentage loss of C was observed in the Free LF for both aggregate size classes. Macro- and microaggregate Free LF C was 80-85% lower in the <1 y stockpiled soils compared to the native soil (Fig. 3b). Macro- and microaggregate iPOM+Sand C was reduced by 60-75% (Fig. 3c). Less change was observed in the macroaggregate Silt+Clay C concentrations (35% reduction) than the microaggregate fraction (52% reduction) (Fig. 3d).

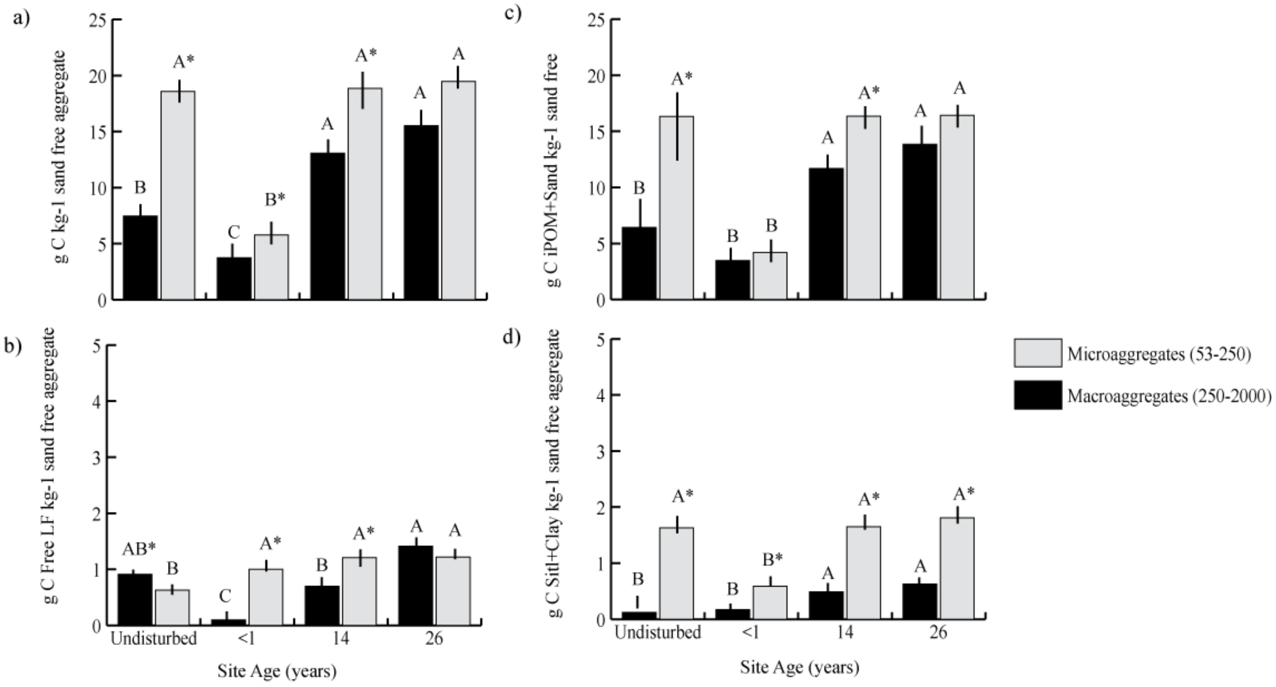

Figure 3. Carbon concentrations in a) macro- and microaggregates b) aggregate associated light fraction (Free LF), c) aggregate associated iPOM+Sand, and d) aggregate Silt+Clay fraction for a C_3 grass chronosequence at Belle Ayr Mine, Gillette, WY For a given soil parameter, columns with a different letter above them are significantly different ($P<0.05$) among site ages. Bars represent one standard deviation, n=3.

Aggregate associated C was also significantly lower in the <1 y old reclaimed soil than in the 26 y old reclaimed site for both macro- and microaggregate size fractions. Reclaimed soil macro- and microaggregate C increased by 3.5 g C kg^{-1} sand free aggregate yr^{-1}. Microaggregate C was higher than macroaggregate C, which has not been commonly observed. Microaggregate MRT for C was only slightly longer (22 yr) compared to macroaggregate MRT (17 yr).

Macroaggregate Free LF C concentrations increased through time, but did not reach concentrations observed in the native site soil. Microaggregate Free LF C increased with each reclaimed site age and was higher than observed concentrations in the native site soil. A majority of aggregate C was found in the iPOM+Sand fraction. Macro- and microaggregate iPOM+Sand C followed similar trends to aggregate C concentrations with reclamation age. Macro- and microaggregate Silt+Clay C concentrations were the same as that observed in the native soil for the 14 and 26 yr old sites.

<u>Organic Carbon Dynamics.</u>

Stable ^{13}C isotope signatures of whole soil, macro- and microaggregate OM were significantly lower in δ^{13}C as reclamation age increased (Fig. 4) in the cool season grass Belle Ayr chronosequence. Whole soil became more depleted in ^{13}C with reclamation age indicating 59% new C. Macroaggregate new C was incorporated more quickly between the <1 and 14 y old site (44% new C) and slowed approaching the 26 y old soil (66% new C). Microaggregate new C was found to be 62% of total organic C between the <1 yr old site and 14 yr old reclamation and did not increase after 26 yr. It is important to consider that coal dust (δ^{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.

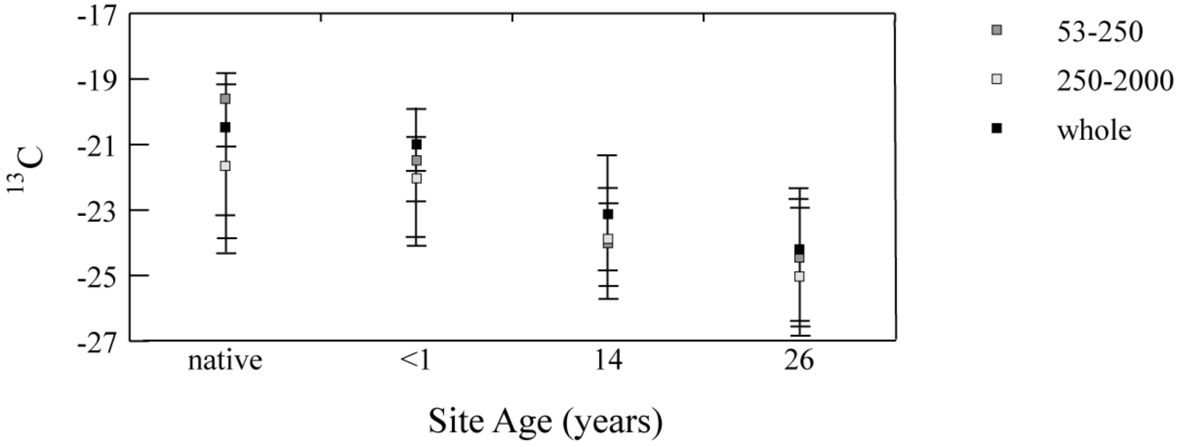

Figure 4. δ^{13}C signatures of whole and aggregate associated organic carbon for a C_3 grass chronosequence at the Belle Ayr Mine, Gillette, WY. Macroaggregates (250-2000 μm), microaggregates (53-250 μm) and whole (total soil). Bars represent one standard deviation, n=12.

Potentially Mineralizable Carbon

Potentially mineralizable carbon assays indicate the metabolically active pool of soil carbon redevelops within 5 years of reseeding in the two reclaimed soils we examined (Data not shown). In soil from the Dave Johnson Mine, 5, 10, and 16 year old reclaimed soils had similar amounts of potentially mineralizable carbon as the nearby undisturbed soil but significantly more than the <1 yr old reclaimed soil (Fig. 5). At the Belle Ayr Mine, only 1.5 year old and < 1 year old reclaimed soils had significantly less available carbon than the undisturbed soil, while the 14 and 26 year old reclaimed soils had similar amounts. No reclaimed soils were found to contain more potentially mineralizable C than undisturbed soils.

Discussion

Lignin contents in SOM and aggregate associated C of reclaimed mine soils in both chronosequences were greater than or equal to that of undisturbed soils. In a recent study on reclaimed mine SOC accumulation in Ohio, Lorenz and Lal (2007) also observed that chemical and biochemical mechanisms were responsible for SOC accumulation in reclaimed mine soils. The minerally protected pool, measured by hydrofluoric acid (HF) treatment of soil, and the biochemical resistant pool, determined by oxidation with sodium persulfate ($Na_2S_2O_8$), constituted up to 70% and 55% of TOC in Ohio mine soils reclaimed to pastures and forests, respectively. Greater concentrations of lignin in TOC of reclaimed soils in our study may be attributed to a combination of soil structure making OM less accessible, selective decomposition of easily decomposable compounds such as polysaccharides resulting in preservation of recalcitrant long chained C, and lack or low activity of lignin-degrading saprotrophic fungi (i.e., basidiomycetes) in the reclaimed site soils (Piccolo, 1996; Almendros *et al.*, 1998; Rana *et al.*, 2006; Wick *et al.*, 2009). Significant (p=0.050) negative correlations between SOC contents and lignin concentrations in SOC in both mine site soils indicated that as organic carbon concentrations decreases in soil, the proportion of lignin in SOC increases due to selective preservation (Ganjegunte *et al.*, 2004). The biochemical recalcitrance of lignin is mainly due to its inherent structural stability, especially rigid alkyl structure, greater aromacity, and lack of appropriate enzymes to breakdown these compounds (Stout *et al.*, 1981; Gleixner *et al.*, 2001; Swift, 2001). In addition to lignin, the proportions in which some of the recalcitrant compounds such as waxes, cutins, suberins, polyphenols, and tannins occur in OM dictate the decomposability of OM (Dai *et al.*, 1996; Girisha *et al.*, 2003; Ganjegunte *et al.*, 2006). These compounds may be present initially in the plant and animal residues added to the soil. Ingram *et al*. (2005) in a study evaluating microbial respiration in reclaimed mine lands in Wyoming reported greater C mineralization rates for reclaimed soils and attributed the differences in soil structural properties, different microbial communities, and preferential decomposition of easily degradable substrates (e.g., carbohydrates) by microbes.

Results of this study demonstrate the potential for soil carbon pools to redevelop rapidly in reclaimed surface mine lands. While physico-chemical protection of clay and greater lignin content of SOC offering resistance to decomposition were responsible for SOC gains in fine-textured soils at the Belle Ayr C_3 grass chronosequence, greater lignin content of OM was the main determinant of SOC dynamics in coarse-textured shrub chronosequence soils at the Dave Johnston mine. There was a net accumulation of SOC in C_3 grass chronosequence at the Belle Ayr mine, which highlighted that when conditions are right, reclaimed mine soils can sequester C at a rapid rate of 0.52 Mg ha^{-1} yr^{-1}. In the shrub chronosequence at the Dave Johnston mine, accumulation of SOC might have been affected by the coarse-textured nature of the soils, which resulted in low adsorption sites available to bind SOC and minimize loss of OC. The C:N ratio of reclaimed mine soils indicated possible contribution (up to 2.0%) to SOC from coal particles in some reclaimed sites. Soil organic C concentrations were greater in surface soils indicating deposition of OM from aboveground vegetation products and plant roots. Vanillyl and syringyl groups made up the bulk of lignin in our chronosequence sites. Increasing $Ac:Al_S$ and $Ac:Al_v$ ratios and declining C:V ratios over time indicated oxidation of lignin to its monomers in reclaimed mine soils. Accumulation of SOC in reclaimed soils on surface mined land should be viewed as an important mechanism by which the coal mining industry can contribute to the reduction in CO_2 concentrations in the atmosphere and at the same time an improvement in soil quality on reclaimed coal mine lands.

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