PHYSICAL PROTECTION OF ORGANIC MATTER IN RECLAIMED COAL MINE SOILS OF SW VIRGINIA[1]

A.F. Wick[2] and W.L. Daniels

Abstract: Particulate organic matter (POM) and aggregate recovery following mining disturbances are important for soil biogeochemical properties and ecosystem function. The objective of this study was to track POM accumulation and aggregation in reclaimed soils following coal mining in southwestern Virginia. A chronosequence of sites was selected based on shifts in vegetation communities with succession, typically occurring between 0-2, 5-7, 16-20, and 38-42 years since reclamation. Undisturbed adjacent forested sites were also sampled. The 0-2 yr old sites were covered with grasses and forbs, the 5-7 yr sites by thick stands of *Lespedeza cuneata*, the 16-20 yr sites predominately with *Festuca arundinaceae* and patches of deciduous trees (*Acer rubrum, Oydendrum arborea*, etc.) and the 38-42 yr old sites with a mix of *Pinus taeda* and deciduous forest with a grass understory. Undisturbed sites predominantly supported mixed Appalachian deciduous forest. Available POM (inter-aggregate) and physically protected (intra-aggregate) forms were determined using a density flotation technique and aggregate size distribution with wet sieving. Inter-aggregate POM did not change across site ages; however, intra-aggregate POM increased significantly between the 5-7 and 16-20 yr old sites and remained unchanged through the 38-42 yr old reclaimed site. Inter-aggregate POM reached levels similar to undisturbed sites, while intra-aggregate POM weights were almost threefold that of undisturbed sites after 16-20 years. By observing just the available POM, we would conclude that reclaimed systems recover to an protected POM, reclaimed system POM storage greatly exceeded undisturbed soil conditions. A positive relationship was observed between small macroaggregates (250-2000 μm) and intra-aggregate POM, suggesting that protection of POM by small macroaggregates in reclaimed systems is extremely important for POM accumulation and subsequently ecosystem function within a period of 20 years. We can also argue the greatest rates of POM accumulation and aggregate formation occur under early succession communities with grasses and forbs rather than under late succession forested communities.

Additional Key Words: microaggregate, organic matter, Appalachian hardwoods, soil quality, sodium polytungstate.

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[1]Paper was presented at the 2009 National Meeting of the American Society of Mining and Reclamation, Billings, MT ***Revitalizing the Environment: Proven Solutions and Innovative Approaches*** May 30 – June 5, 2009. R.I. Barnhisel (Ed.) Published by ASMR, 3134 Montavesta Rd., Lexington, KY 40502.

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Proceedings America Society of Mining and Reclamation, 2009 pp 1564-1582
DOI: 10.21000/JASMR09011564
http://dx.doi.org/10.21000/JASMR09011564

Introduction

Research on soil aggregation in reclaimed mined lands has generally focused on soil stability (or resistance to erosion) rather than influences of aggregation on reclaimed soil structure and function, i.e. organic matter (OM) protection and dynamics (Vogel, 1987). Structurally, aggregation directly influences soil properties such as, but not limited to, bulk density and pore size distribution (Hillel, 1982) which directly influence soil water movement and gas exchange. 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 particulate organic matter (POM), which provides a metabolic energy pool for microbes and macronutrients for plants, and is therefore one regulator of microbial decomposition and nutrient availability (Essington, 2004). Reclaimed mine sites exhibit primary successional processes and present a unique system for detailed examination of soil processes such as aggregation and OM protection.

Mining activities greatly reduce surface soil OM through either: (1) soil horizon mixing and aggregate disruption during topsoil salvage prior to mining and return with reclamation (Ussiri and Lal, 2005; Lorenz and Lal, 2007, Wick et al., 2009) or (2) the inability to salvage topsoil material prior to mining resulting in the utilization of mine spoils in reclamation. In reclamation where topsoil is salvaged and returned, most macroaggregation (250-8000 μm) is destroyed because roots and fungal hyphae holding macroaggregates together are disrupted. Microaggregates (53-250 μm), which form and stabilize in the interior of macroaggregates, are then released into the soil (Wick et al., 2009). A soil recently disturbed by mining (with associated topsoil salvage, storage and replacement) will typically have higher proportions of microaggregates and lower proportions of macroaggregates than an undisturbed soil, which in addition to releasing once protected POM for microbial decomposition, can also greatly influence soil physical properties (Six et al., 1998; Wick et al., 2009). In the Appalachian coal mining region studied here, topsoil salvage and return are seldom employed and topsoil substitutes formed out of fresh overburden materials are the dominant substrate upon which primary successional processes are supported (Haering et al., 2004). Rates of aggregate formation and OM accumulation in soils developing from mine spoils are currently unknown and of interest due to the functional importance of soil aggregation and OM for soil biogeochemical cycling.

Soil aggregate recovery, its relationship with POM accumulation, and the rate of recovery towards pre-disturbance conditions in drastically disturbed mined lands are important factors for ecosystem function and reclamation success (Lyle, 1987; Jastrow et al., 1998). Objectives of this research were to: (1) quantify aggregate size distributions through time, (2) quantify changes in inter- and intra-aggregate POM with time, and (3) look for relationships between aggregation and POM. We hypothesized that: (1) Large and small macroaggregates will increase with time since reclamation; (2) inter-aggregate POM will increase drastically following plant establishment; (3) intra-aggregate POM will be highest in the oldest reclaimed sites; and (4) aggregation will be highly correlated with whole soil POM content.

Materials and Methods

Study Sites and Field Sampling

A chronosequence of sites at the Powell River Project research area in Wise County, Virginia (Haering et al., 2004) was selected based on locally documented shifts in vegetation communities with succession (Li and Daniels, 1994), which tend to occur between 0-2, 5-7, 16-20, and 38-42 years since reclamation in this area. Undisturbed adjacent sites were also sampled to serve as a reference for pre-mining forested conditions (Fig. 1). Though spatial variability within each site in the chronosequence can be greater than temporal variability represented by the chronosequence; use of this technique is necessary for the evaluation of reclamation treatments. With this in mind, as many factors as possible were held constant to allow for the isolation of "time" on soil aggregation and OM accumulation. All sites were located over mine soils derived from Wise Formation sandstone/siltstone mixes and all except the oldest sites had been reclaimed by the same coal company (Red River Coal) using similar revegetation methods. The 0-2 yr old sites were covered with reestablished grasses and forbs, the 5-7 yr sites by thick stands of *Lespedeza cuneata*, the 16-20 yr sites predominately with *Festuca arundinaceae* and patches of invading deciduous trees (*Acer rubrum, Oydendrum arborea*, etc.) and the 38-42 yr old sites with a mix of planted *Pinus taeda* and deciduous forest with a grass understory. Trees on the 38-42 yr old sites were planted in 1980; however, the soil development in these sites reflects the age assigned. Undisturbed sites predominately supported mixed Appalachian deciduous forest with a mixed forb/grass understory. Unfortunately, to capture the 16-20 yr shift in plant communities, a subset of sites receiving biosolids had to be sampled. Though this management technique can greatly influence soil OM (a major reason why biosolids are used as a soil amendment), we felt it was necessary to sample sites in this age range. Figure 2 shows vegetation differences across site ages.

Four samples were carefully collected from three replicates within each site age with a trowel from each site either along a 4 m long transect or around a soil pit from the 0-5 cm depth where differences in POM would be most prevalent among site ages. Samples were kept cool to minimize microbial influences on samples until they were air dried and sieved in the lab.

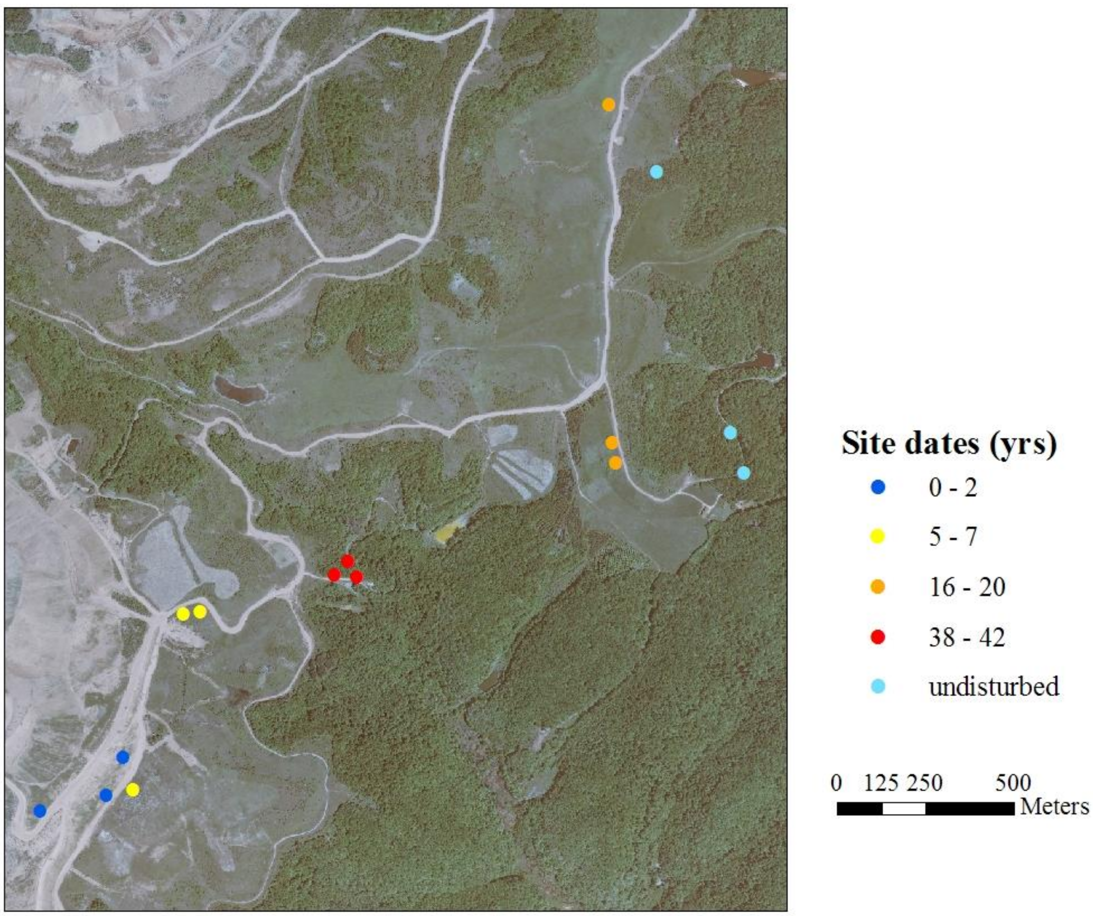

Figure 1. Site locations presented by site age in the Powell River coal fields of southwestern Virginia. Geology and mine soils of area shown here are documented by Haering et al. (2004).

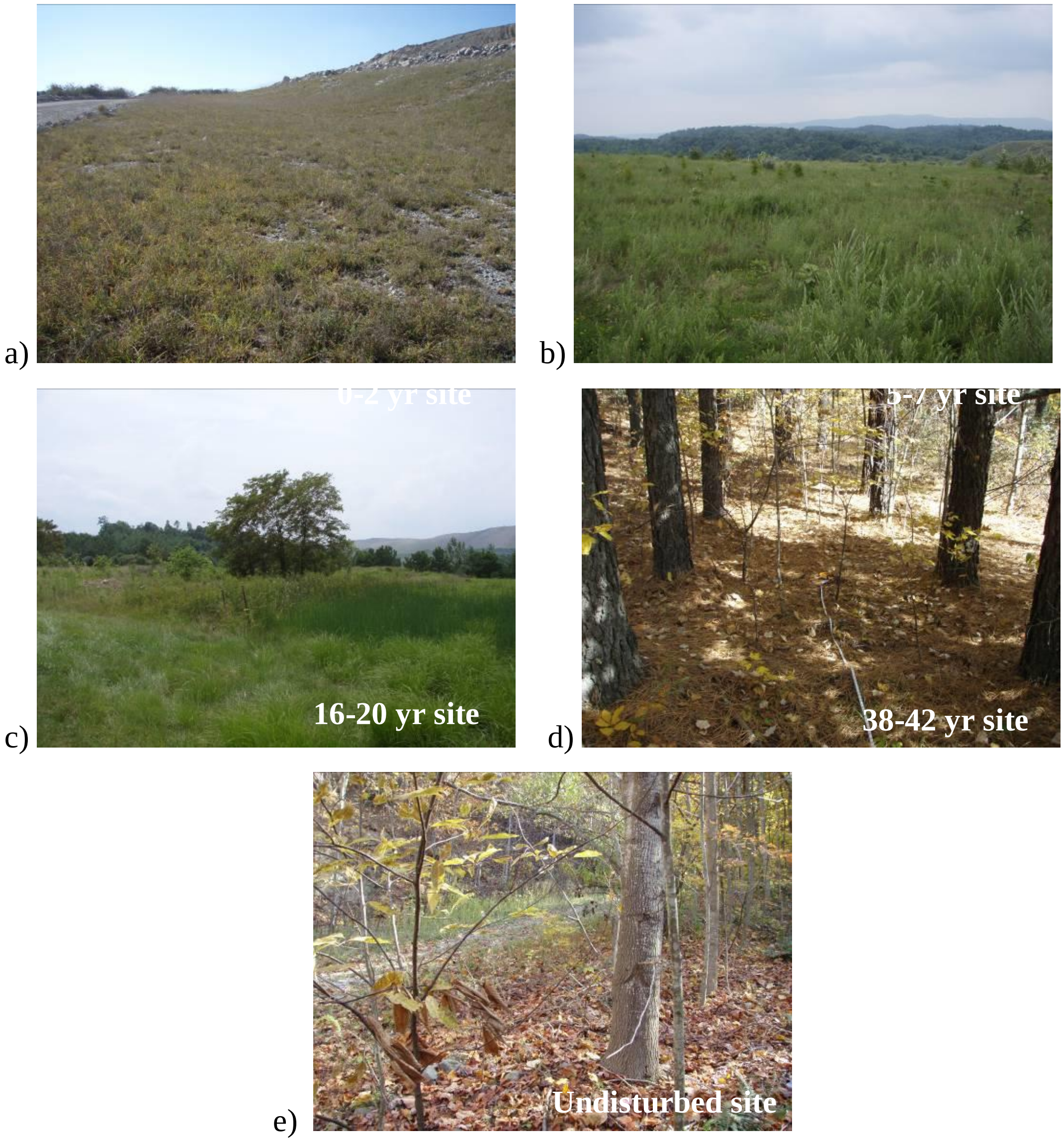

Figure 2. Vegetation found at each site age of a chronosequence established in the coal fields of southwestern Virginia. a) 0-2 yr site, b) 5-7 yr site, c) 16-20 yr site, d) 38-42 yr site, and e) undisturbed site.

General Soil Properties

All samples were air dried and dry sieved to 8000 µm to remove large roots and break apart soil clods while leaving structure <8000 µm intact. A subsample was sieved to 2000 µm for basic soil analyses of electrical conductivity (EC) and pH 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 analyzing EC and pH, respectively.

Soil particle size distribution was determined with the pipette method on a composite of the four samples collected from each site (NRCS, 2004).

Aggregate Size Distribution

Water stable aggregate size distribution of soil was determined using a wet sieving protocol described by Six et al. (1998) on all 8000 μm sieved samples. In summary, 50 ± 0.02 g of air dried soil were submerged in deionized water for 5 min at room temperature on a 2000 μm sieve (20.5 cm in diameter). Water stable large macroaggregates (2000-8000 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 250 μm sieve and the above process repeated for small macroaggregates (250-2000 μm). Material remaining on the sieve was again transferred to a 53 μm sieve and the above process repeated for microaggregate structure (53-250 μm). Material collected from each sieve (2000-8000 μm, 250-2000 μm and 53-250 μm) was dried at 55˚C until a constant weight was achieved. Material passing through the 53 μm sieve was considered to be free silt+clay and though it was not collected, a weight for this fraction was determined by subtracting aggregate masses from the 50 g sample weight.

Sand corrections were determined on all samples according to Denef et al. (2001) for clarity when comparing across sites of different soil textures. Five mL of sodium hexametaphosphate and 10 mL of water were added to separate 5 gram subsamples of soil. Samples were shaken on a reciprocal shaker for 18 h and sieved with nested 2000 μm (large macroaggregates), 250 μm (small macroaggregates) and 53 μm sieves (microaggregates). Samples collected on each sieve were dried and weighed to determine a sand correction value (weight basis).

Density Floatation

Particulate OM analysis (for both inter- and intra-aggregate POM) was conducted according to methods described by Six et al. (1998). Whole soil samples (5 g) 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 (inter-aggregate available, 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. Twelve 6 mm glass beads and 30 mL 1.85 g cm^{-3} density SPT were added to the material remaining in the centrifuge tube (intra-aggregate POM, sand, silt and clay) and samples were shaken overnight. Following shaking, sides of the centrifuge tubes were rinsed with 10 mL SPT and centrifuged for 60 min at 2,500 rpm and floating material (intra-aggregate POM, iPOM) 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. Deionized water was added (30 mL) to the remaining soil pellet (sand, silt and clay), sample was shaken and sides were rinsed with 10 mL water. Samples were then centrifuged for 60 min at 2,500 rpm. This process was completed three more times to fully rinse and recover the SPT from the samples. The remaining soil pellet was discarded.

Statistical Analyses

One way analysis of variance was used to determine differences among reclaimed sites followed by t-tests for separation of means. Relationships among variables were evaluated with Pearson correlations (SigmaPlot, 2008). Undisturbed sites were not included in statistical analyses and were used for comparison to soil conditions prior to disturbance. All statistical analyses were accomplished at $P<0.05$.

Results

As expected, EC was low for all site ages (<0.60 dS m^{-1}; Table 1), suggesting soil aggregation was not affected by any minor differences in soil salinity among site ages. Soil pH was significantly higher in the 0-2 yr sites compared to the oldest reclaimed sites (38-42 yrs), which might have affected microbial communities involved in initial aggregate formation and stability. The fresh overburden in this area contains trace carbonates (1 to 2%) which buffer the pH to > 7.0 in young mine soils and then decreases with mine soil age and/or the inclusion of significantly preweathered overburden in the older mine soils (Haering et al., 2004). Soil textures ranged from sandy loam to loam among site ages. The 5-7 yr site was significantly higher (18%) in clay and lower in sand (33%) compared to the other sites (12-14% clay and 48-58% sand).

Table 1. Soil electrical conductivity (EC), pH and texture for a chronosequence of sites sampled in the coal fields of Southwestern Virginia. Significant differences shown across reclaimed site age (P<0.05); values in parentheses are one standard error of the mean. Undisturbed site EC, pH and soil texture were not included in the statistical analysis.

Site Age	**EC**	**pH**	**Sand**	**Silt**	**Clay**
(yrs)	**(mmhos cm^{-1})**			**%**	
0-2	0.36 (0.09)	8.08 (0.10) a	58 (10) a	30 (8.2) b	12 (2.5) b
5-7	0.57 (0.24)	6.27 (1.1) ab	33 (7.8) b	49 (6.3) a	18 (1.6) a
16-20	0.58 (0.13)	5.93 (0.49) ab	56 (8.7) a	31 (8.3) b	13 (3.7) b
38-42	0.32 (0.03)	5.38 (0.41) b	48 (3.7) a	38 (2.4) a	14 (1.3) b
Undisturbed	0.44 (0.14)	5.75 (1.16)	59 (8.3)	28 (9.1)	13 (0.73)

Soil Aggregate Structure

There were more large macroaggregates in the 5-7 (0.44 g aggregate g^{-1} soil) and 38-42 yr (0.48 g aggregate g^{-1} soil) than in the 0-2 (0.21 g aggregate g^{-1} soil) and 16-20 yr (0.31 g aggregate g^{-1} soil) old sites. Small macroaggregate proportions were similar across site ages (Fig. 3). Microaggregates were higher in the 0-2 yr old site (0.18 g aggregate g^{-1} soil) than the other older reclaimed sites (ranging from 0.06 to 0.11 g aggregate g^{-1} soil). Free silt+clay weights were higher in the newly disturbed site (0-2 yrs) than other reclaimed sites. The undisturbed soil contained equal parts large and small macroaggregates (0.30 and 0.31 g aggregate g^{-1} soil, respectively), and half as many microaggregates as the 0-2 yr old soils (0.09 g aggregate g^{-1} soil). Aggregate size distributions in the undisturbed sites resembled those in the 16-20 yr old sites.

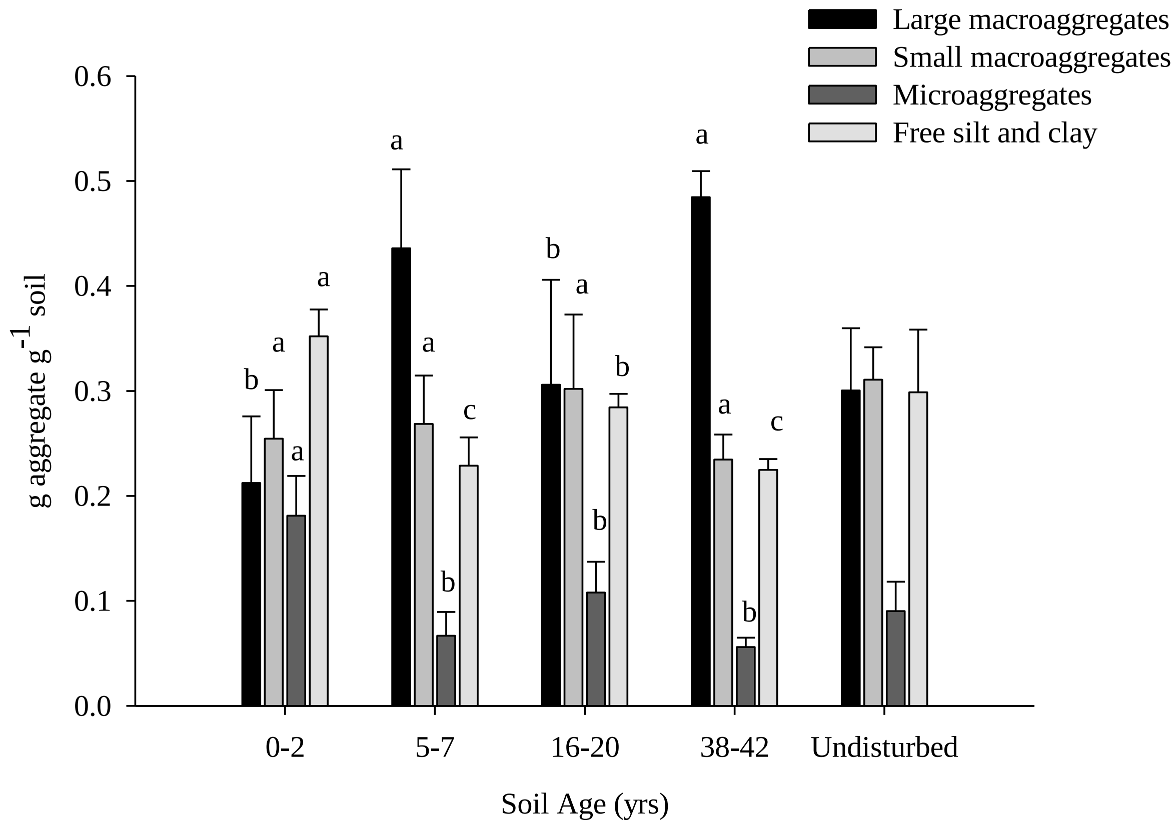

Figure 3. Soil aggregate size distributions for a chronosequence of reclaimed soils in the coal fields of southwestern Virginia. Undisturbed site aggregate size distributions are shown for reference. Error bars represent one standard error of the mean and letters indicate significant differences among site ages for each aggregate size class and free silt and clay across reclaimed soil ages only.

Particulate Organic Matter

Though statistically inter-aggregate POM weights were similar across site age, there was an increasing trend from the 0-2 yr (8.85 g POM kg^{-1} soil) to the 16-20 yr site (67.5 g POM kg^{-1} soil; Figs. 4 and 5). Intra-aggregate POM was higher in the 16-20 and 38-42 yr old sites (158 g POM kg^{-1} soil and 57 g POM kg^{-1} soil, respectively) than younger reclamation ages (ranging from 30.9 to 38.9 g POM kg^{-1} soil). Undisturbed sites had higher inter-aggregate POM (72.3 g POM kg^{-1} soil) and similar amounts of intra-aggregate POM (35.7 g POM kg^{-1} soil) as the newly reclaimed sites (<10 yrs).

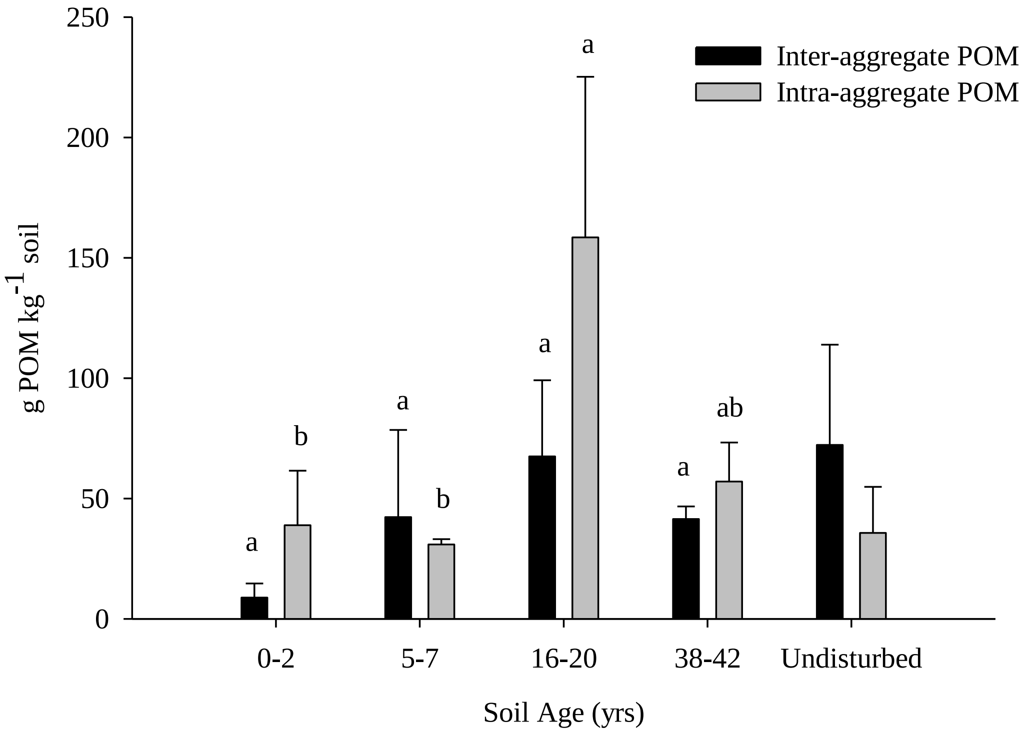

Figure 4. Inter- and intra-aggregate particulate organic matter (POM) weights for a chronosequence of reclaimed sites in the coal fields of Southwestern Virginia. Undisturbed site POM is shown as a reference. Error bars represent one standard error of the mean and letters indicate significant differences among site ages for each POM fraction.

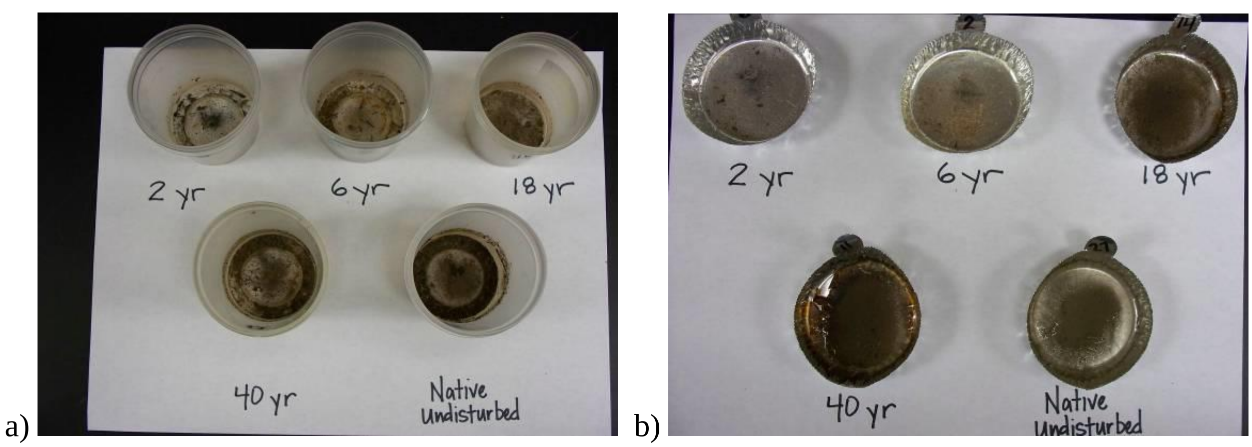

Figure 5. Particulate organic matter (POM) density fractionations for a chronosequence of reclaimed sites in the coal fields of Southwestern Virginia. Undisturbed is shown as a reference. a) inter-aggregate POM and b) intra-aggregate POM.

Relationships among Variables

Inter- and intra-aggregate POM were highly correlated (R^2 = 0.71, P = 0.01) indicating a relationship between unprotected OM available for microbial decomposition and that protected by aggregate structure and stored. Intra-aggregate POM was correlated with small macroaggregate structure (R^2 = 0.61, P = 0.04) rather than the two other size classes, making small macroaggregates most important for protecting POM. Microbial biomass carbon (used as an estimate of microbial recovery in reclaimed soils) data collected by Clayton et al. (2009) for these same series of sites was related to both intra-aggregate (R^2 = 0.81, P = 0.001) and inter-aggregate POM (R^2 = 0.70, P = 0.01). As both types of POM increase, microbial biomass carbon also increased. Increases in large macroaggregates had a corresponding decrease in microaggregates indicating aggregate hierarchy (where microaggregates are formed, stabilized and incorporated in macroaggregates; R^2 = -0.95, P < 0.0001).

Discussion

Soil aggregate and OM dynamics of soils developing on mine spoil material were well represented in this chronosequence of sites. First, the typical distributions of macroaggregates, microaggregates and free silt+clay following disturbance were observed in the 0-2 yr sites. This has been consistently found in other reclaimed soils following topsoil salvage (Malik and Scullion, 1998; Wick et al., 2007; Wick et al., 2009) as well as agricultural soils (Six et al., 1998; Jastrow et al., 1998; Grandy and Robertson, 2007). Macroaggregation then peaked in the 5-7 yr and again in the 38-42 yr old reclamation, possibly indicating both a reliance of macroaggregation on clay content (in the 5-7 yr old site) and a healthy turnover of macroaggregates (as shown in the 38-42 yr old site; Jastrow and Miller, 1998; Six et al., 1998). Higher clay contents generally correlate with higher aggregate stability (Kemper and Koch, 1966), where the reorientation of clay particles and binding of root exudates to clay by wet-dry cycles increases macroaggregation (Reid and Goss, 1982). The lower macroaggregation in the 38-42 yr old reclaimed site is typical of naturally occurring aggregate turnover, where macroaggregates have been found to be stable for a period of <50 yrs (Six et al., 2002).

Interestingly, aggregation was not related to microbial biomass carbon; however, inter-aggregate POM weights were correlated to microbial biomass carbon and were similar across reclamation ages. Intra-aggregate POM was related to small macroaggregates, indicating the importance and stability of this size fraction for POM accumulation in reclaimed soils. Aggregate and POM trends across the site ages could be explained by a combination of factors; (1) successional plant community influences, (2) naturally occurring aggregate turnover, and (3) biosolids effects in the 16-20 yr sites.

Plant community types are extremely important for aggregate formation and OM accumulation in both protected and unprotected aggregate pools (Carter et al., 1994; Eviner and Chapin, 2002; Ehrenfeld et al., 2005; Wick et al., in press). Root entanglement around soil particles, concurrent secretion of carbohydrates as roots wrap around and bind soil particles together, and wet-dry cycles are all essential processes for aggregate formation and stabilization (Gale et al., 2000). Additionally, microbial associations with various types of plants (mycorrhizal fungi etc.) influence aggregate formation and stability (Jastrow, 1987; Ehrenfeld et al., 2005). Perennial grasses and legumes with fine root systems and relatively rapid decomposition rates have been associated with higher aggregate stability than agricultural crops (Jastrow, 1987). Wick et al. (2007) observed higher aggregation under annual forbs than communities dominated by perennial grasses after 14 years of mined land reclamation in the western USA. Lower aggregation but higher OM content has been observed under shrubs with coarse roots compared to grasses with fine roots (Liao et al., 2006; McClaran et al., 2008; Wick et al., in press). Obviously, each plant community is unique in its influence on aggregation and contributions of OM.

Successional shifts in plant communities observed in this study likely influenced soil aggregation and OM. Between 2 and 5 years, grasses and forbs dominating the sites probably contributed to the observed increase in macroaggregation, incorporation of microaggregates within macroaggregates and POM accumulation from root sloughing and carbon allocation shifts from above- to below-ground biomass. *Lespedeza* likely maintained aggregation in the 5-7 yr age range because of its fungal associations (Rothwell, 1984). Fungal hyphae wrap around soil particles and play a dominant role in macroaggregate formation (Jastrow and Miller, 1998). Additionally, the high lignin content found in the roots of *Lespedeza* could facilitate the persistence and accumulation of soil OM (Rothwell, 1984). Gangegunte et al. (2009) have found higher lignin contents in reclaimed compared to undisturbed soils and attributed the rapid accumulation of OM and thus higher carbon storage in reclaimed soils to lignin contributions.

Particulate OM in the *Festuca* dominated 16-20 yr old was probably a combination of residual material from the *Lespedeza* communities and *Festuca* inputs. *Lespedeza* does not form pure stands when biosolids are applied but remains a significant component of the plant community for up to 10 years. Though *Festuca* allocate less than 10% of C acquired from photosynthesis into root development (Belanger et al., 1994), root turnover still contributed to POM pools in these sites. Tree dominated communities found on the 38-42 yr old sites should have lower macroaggregation from coarse root penetration and higher amounts of POM compared to the other sites because of the lower bioavailability of woody litter (especially from *Pinus* species), and reduced microbial activity in the slightly more acidic soils (John et al., 2005; Yamashita et al., 2006). Though these parameters were not observed in this study, we can speculate that microbial root associations were influencing POM accumulation and aggregate formation in these sites and that possibly older reclaimed sites dominated by tree species would have more POM. Regardless, early successional plant communities appeared to have had the greatest influence on aggregation and POM accumulation in reclaimed soils.

In addition to plant influences, soil aggregates naturally turnover with time which controls soil OM release for decomposition and storage (Six et al., 1998; Leij et al., 2002; Plante and McGill, 2002; De Gryze et al., 2006). Disturbances accelerate the turnover of aggregates and therefore expose more POM for decomposition. Typically, the mean residence time (MRT) of POM protected by large and small macroaggregates has been reported as 42 ± 18 years while the MRT for microaggregates is 320 ± 80 years in undisturbed soils (Skjmestad et al. 1993; Six et al. 2002). As aggregates turnover, new ones are formed to create some consistency in aggregate size distributions with time. We speculate that observed trends in aggregate recovery between the 5-7 and 16-20 yr site were partially due to the higher clay content in the 5-7 yr site (as explained earlier) and that a more gradual increase in macroaggregation with time until aggregates turnover in the 38-42 yr site would have been observed if the sites were more similar in clay content. It is possible that both aggregation and OM were at their peak within the 16-20 yr time period and decreased with aggregate turnover around 40 yrs.

Perhaps more importantly, the application of biosolids to the 16-20 yr old sites could have influenced trends observed in this study. Previous studies on the influence of biosolids applications on soil aggregate structure found: (1) aggregate stability and OM content was improved by multiple applications of biosolids (Mitchell, 1978), and (2) a high initial response to biosolids followed by lower aggregation after a single biosolids application (Metzger et al., 1987; Malik and Scullion, 1998). There is obviously a tight relationship between stimulation of microbial communities and supply of OM to serve as a nucleus for aggregate formation when biosolids are applied. If effects of single applications of biosolids diminish with time, then we might expect the high POM weights in the 16-20 yr old sites where biosolids were applied to be a function of "time"; however, if the initial biosolids application is still influencing aggregation and POM, then this level of POM would be inflated. Preliminary data collected by Wick and Daniels (unpublished) from a long-term study site located at the same mine, where biosolids treatments (56 Mg ha^{-1}) were compared to control treatments (overburden material) after 30 years of soil development, indicated similar inter- and intra-aggregate POM weights in the biosolids treatment as the control treatment. However, inter-aggregate POM was much higher under the biosolids treatment (107.6 g POM kg^{-1} soil) than the control (58.85 g POM kg^{-1} soil) and the control intra-aggregate POM was slightly higher (34.12 g POM kg^{-1} soil) than the biosolids treatment (29.28 g POM kg^{-1} soil). Research on the same long-term experiment collected by Bendfeldt et al. (2001) indicated similar aggregate stability and amounts of soil OM between the biosolids and control treatments after 16 years of soil development. Clearly, more research is needed on the long-term effects of amending disturbed soils with biosolids on OM persistence and aggregate protection of OM.

Conclusions

Soil formation on fresh overburden materials follows similar recovery trends with time as reclaimed areas where topsoil salvage and replacement is practiced and following abandonment of agricultural fields (were a majority of aggregate and POM research is currently being done). Aggregate formation occurs quickly under early successional reestablished plant communities and is followed by an increase in POM. There is a lag time between the two parameters indicating a tight relationship and importance of aggregation for OM accumulation. Both parameters recover to or exceed an undisturbed soil condition after a period of 20 years. In these soils, small macroaggregates contribute the most to POM protection of the three aggregate size classes. Defining long-term aggregate and POM trends in these soils is important for management of reclaimed mine lands as well as determining reclamation success.

Additional work with the samples collected from these sites is needed to fully understand long-term aggregate and OM dynamics in eastern reclaimed soils. For instance, carbon and nitrogen need to be quantified as a proxy for OM, the OM needs to be characterized to determine recalcitrant vs. available pools, and the physicochemical binding of OM to silt and clay particles needs to be evaluated to determine long-term carbon storage, to name a few. There is great potential with these sites to relate microbial communities to the aggregate and OM dynamics and to understand shifts of interlinked biological and physical soil properties with time.

Acknowledgements

We want to thank Whitney Nash and Hannah Clayton for field assistance and Julie Burger and Dan Johnson for lab assistance. We also thank Pat Donovan for assistance with site location figure. This project was funded by the Powell River Project, via Virginia Polytechnic Institute and State University.

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