SOIL AGGREGATE, ORGANIC MATTER AND MICROBIAL DYNAMICS UNDER DIFFERENT AMENDMENTS AFTER 27 YEARS OF MINE SOIL DEVELOPMENT [1]

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Abstract: Physical and biological properties of soils developing from spoil material following surface coal mining in southwest Virginia are poorly understood. Additionally, the effects of various types of soil amendments such as sawdust, topsoil or biosolids on long-term soil development are lacking in the current literature. The objective of this study was to examine water stable aggregation, organic matter (OM) content and microbial biomass in a long-term experiment (27 yr) where various types (control-CON, topsoil-TS, sawdust-SD, and biosolids-B) and rates of soil amendments (biosolids: B-22, B-56, B-112 and B-224 Mg ha^{-1}) were applied in 1982. Treatments were replicated four times in a randomized complete block design. Small macroaggregates (250-2000 μm) were higher on the B-224 rate plots compared to other treatments, while there were no differences in large macroaggregates (2000-8000 μm) or microaggregates (53-250 μm) among treatments. Aggregate associated OM, as indicated by carbon (C) and nitrogen (N) concentrations, was highly variable among treatments. Biosolids treatments were clearly higher in total aggregate C and N relative to the CON, TS, and SD treatments; however, these differences were not significant for each aggregate size class due to the variability observed among replicates. There were no significant differences in aggregate C and N among biosolids application rates after 27 years of soil development. However, microbial biomass C was higher in all biosolids treatments compared to the CON, TS, and SD treatments and was slightly higher on the B-56 treatments relative to other biosolids treatments. Despite the large variability in soil development observed in these relatively small research plots, higher rates of biosolids amendments slightly improved macroaggregate structure while application rates between 22 and 56 Mg ha^{-1} appeared to improve aggregate associated C and N concentrations and soil biological properties.

Additional Key Words: macroaggregate, microaggregate, organic matter, Appalachian coal fields, soil quality, microbial biomass, southwest Virginia.

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List of abbreviations

B-22, biosolids treatment 22 Mg ha-1; **B-56** biosolids treatment 56 Mg ha-1; **B-112**, biosolids treatment 112 Mg ha-1; **B-224**, biosolids treatment 224 Mg ha-1; **C**, carbon; **CON**, control treatment; **EC**, electrical conductivity; **F**, fumigated; **Free LF**, inter-aggregate particulate organic matter; **iPOM**, intra-aggregate particulate organic matter; **MBC**, microbial biomass carbon; **N**, nitrogen; **NF**, non-fumigated; **OM**, organic matter; **POM**, particulate organic matter; **SD**, sawdust treatment; **SPT**, sodium polytungstate; **TOC**, total organic carbon; **TS**, topsoil treatment.

Introduction

Very few long-term (> 10 yr) research experimental sites exist on reclaimed mine lands and of those sites, even fewer are considered "maintained". In light of continued evaluation of reclamation treatments and management practices, these sites are extremely valuable and provide insight into determining success well beyond bond release periods which are five years in the eastern United States (SMCRA, 1977). One of the longest continually maintained experiments (Controlled Overburden Placement experiment) is located on a surface coal mine in southwestern Virginia (Wise County) and is managed by the Powell River Project and faculty in the departments of Crop and Soil Environmental Science and Forestry at Virginia Tech (Roberts et al., 1988a,b). This site has been maintained and monitored for 27 years and allows us to address issues related to the effects of soil amendments on soil development and vegetation establishment as well as answer questions related to reclaimed mine land carbon (C) storage potential. There are multiple facets to this experiment; however, in this study, the effects of soil amendments (topsoil and sawdust additions as well as various rates of biosolids additions) on soil biogeochemical properties were of particular interest.

Long-term development of soil structure/aggregation, accumulation and persistence of organic matter (OM) and recovery of microbial communities continues to draw attention in the scientific community. Although these parameters are well understood in agricultural scenarios (Six et al., 1998; Jastrow et al., 1998; Grandy and Robertson, 2007), they are poorly understood in re-developing soils following mining disturbances. Structurally, aggregation directly influences soil properties such as, but not limited to, bulk density and pore size distribution which directly influence soil water movement and retention and gas exchange (Hillel, 1982).

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 biological functions 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; Coleman et al., 2004). Reclaimed coal mine sites, especially those in the central Appalachians, present a unique system for detailed examination of soil processes such as aggregation, OM and microbial dynamics because these mine soils are typically forming directly from blasted rock overburden with limited topsoil and other soil amendment additions (Haering et al., 2004).

The objective of this research was to quantify aggregate size distributions, POM C and nitrogen (N) concentrations and microbial biomass across a range of mine soil amendment treatments after 27 years. We hypothesized that aggregation, inter-aggregate and intra-aggregate POM and microbial biomass would be higher in mid-rate biosolids plots (56 and 112 Mg ha^{-1}) relative to other treatments.

Materials and Methods

Study Sites and Field Sampling

The Controlled Overburden Placement experiment consists of mine soils derived from Wise Formation (upper middle Pennsylvanian) sandstone/siltstone mixes. Various types (control-CON, topsoil-TS, sawdust-SD, and biosolids-B) and rates of soil amendments (biosolids: B-22, B-56, B-112 and B-224 Mg ha^{-1}) were applied over a consistent mix of sandstone/siltstone in 1982 and replicated four times in a randomized complete block design (Fig. 1). Greater detail on initial spoil and amendment properties is provided by Roberts et al. (1988a, b). The CON treatment consisted of fertilized mixed spoil, TS received 30 cm of limed, fertilized and mixed A, B, C and Cr horizon material, SD received 112 Mg ha^{-1} of incorporated hardwood sawdust + added N fertilizer to offset anticipated immobilization. The biosolids applied were municipal secondary cake material removed from sand drying beds in Big Stone Gap, VA. The plots were originally seeded to tall fescue (*Festuca arundinacea* Schreb.) but over the years, a diverse assemblage of other grasses, forbs, vines and limited woody shrubs invaded the plots. Half of each plot was also maintained in planted trees, but not sampled for this project.

Two samples from each plot were carefully collected in 2009 from the 0-5 cm depth by pushing aside all surface residues and sampling mineral soil (A horizon) with a trowel. Differences in aggregation, OM and microbial biomass are most prevalent in surface soils among treatments (Grandy and Robertson, 2007) and significant pedogenesis had been noted in surface depths by Haering et al. (1993) for these particular mine soils. Samples were kept cool (5˚C) to minimize microbial influences on samples. In the lab, half of each sample was air dried for aggregate and OM analyses and the other half was refrigerated for microbial biomass analysis. All samples were gently sieved to 8000 μm to remove large roots and break apart soil clods while leaving structure <8000 μm intact.
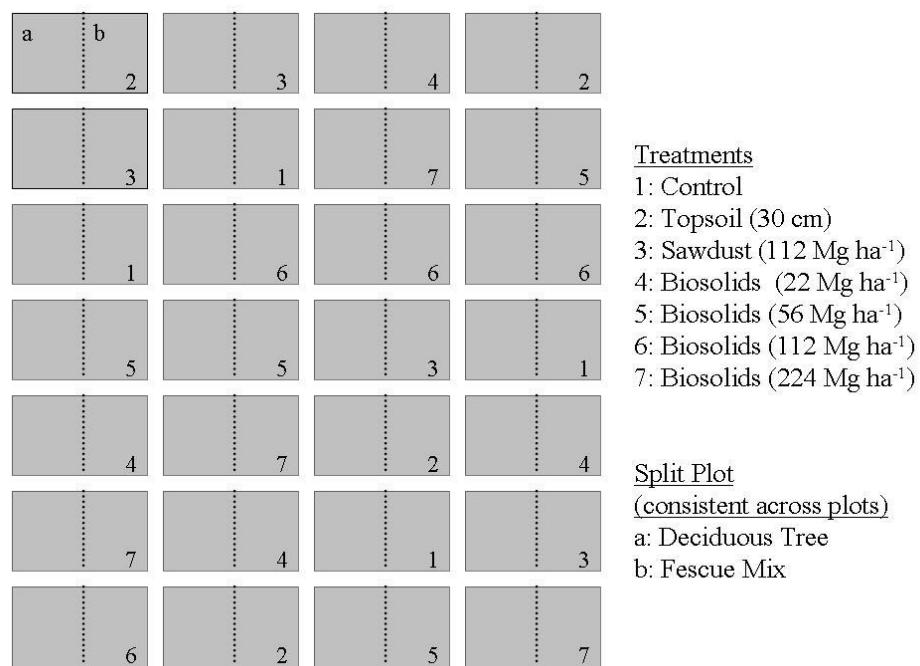

Figure 1. Treatments in the controlled overburden placement experiment
at the Red River Coal Mine, Wise County, Virginia.

General Soil Properties

General soil property analyses were conducted and reported by Nash et al., 2010. Briefly, samples were 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 two samples collected from each plot (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 non-aggregated free silt+clay and was not collected.

Sand corrections were determined on all samples according to Denef et al. (2001) for clarity when comparing across plots 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 sample was sieved to 53 μm to separate the sand from the silt+clay fraction (organo-mineral). Both fractions were dried and weighed.

Carbon and Nitrogen Analysis

Carbon and N concentrations were determined on whole soil samples, all aggregate size fractions and POM fractions via dry combustion (Elementar CNS analyzer, Hannau, Germany). Concentrations were determined for each aggregate and POM fraction sample on a sand free basis (Elliot et al., 1991).

Microbial Biomass Carbon

Chloroform fumigation-extraction was used to determine soil microbial biomass carbon (MBC; Coleman et al., 2004; von Luetzow et al., 2007). Briefly, 10 g of field-moist soil were fumigated (F) with chloroform for 48 h and extracted through a Whatman #42 filter with 50 mL 0.25 M K_2SO_4 (Haney et al., 2001). Non-fumigated (NF) samples were also extracted in this manner. Solutions collected from the extraction were analyzed with a Total Organic Carbon (TOC) Analyzer (Sievers 900, Boulder, CO) within 24 hours of extraction. A dry weight correction factor was applied to TOC values. Equation 1 was used to determine MBC:

$$\text{MBC (g C kg}^{-1}\text{ soil)} = \frac{\text{TOC (F)} - \text{TOC (NF)}}{\text{Kc}} \qquad (1)$$

where Kc = 0.38 (von Luetzow, 2007)

Statistical Analyses

One way analysis of variance was used to determine differences among treatments followed by t-tests for separation of means (SigmaPlot, 2008). Statistical analyses were accomplished at $P < 0.05$ or $P < 0.10$ where specified.

Results

<u>General Soil Properties</u>

   Electrical conductance (0.22 - 0.36 mmhos cm^{-1}) and pH (5.97 – 6.23) were similar across treatments (Table 1; Nash et al., 2010). All soils were classified as sandy loams with sand percentages ranging from 52-62%, silt from 28-38% and clay from 9-20% across treatments. Total C concentrations were significantly lower in the TS treatment relative to all other treatments and total N was generally lower on non-biosolids treatments than plots receiving biosolids (with the exception of the 56 Mg ha^{-1} application rate). For more information on general soil properties and change in these properties with soil development, refer to Nash et al., 2010.

<u>Soil Aggregate Structure</u>

   Soil aggregate size distributions were very similar across treatments (Fig. 2; P=0.817). Total aggregation was higher than 50% of total sand free soil across all treatments and was dominated by macroaggregates (>250 μm; contributing >95% of the total aggregation). Significantly higher small macroaggregation was observed on the B-224 treatment relative to the CON treatment (P=0.041), while other size classes were similar across all treatments.

Table 1. Soil electrical conductivity (EC), pH, texture, bulk density (BD), total carbon (C) and total nitrogen (N) concentrations for various treatments (control, topsoil-30 cm, sawdust-112 Mg ha^{-1}, biosolids-22 Mg ha^{-1}, Biosolids-56 Mg ha^{-1}, Biosolids-112 Mg ha^{-1}, and Biosolids-224 Mg ha^{-1}) at the controlled overburden placement experiment 27 years after treatment application. Significant differences are shown across treatments with different letters ($P<0.05$) and standard deviations from the mean are presented in parentheses, n=4 (Nash et al., 2010).

| Treatment	EC	pH	Sand	Silt	Clay	Total C	Total N
	(mmhos cm^{-1})			%		(g kg-1)	
Control	0.29 (0.07)	6.23 (0.18)	53 (2.99) b	35 (3.78)	12 (5.07)	55.0 (6.6) a	3.18 (0.55) b
Topsoil	0.22 (0.07)	6.21 (0.20)	62 (2.38) a	28 (0.500)	10 (2.22)	38.0 (3.2) b	2.74 (1.1) b
Sawdust	0.25 (0.08)	6.19 (0.27)	52 (2.06) b	28 (16.4)	20 (16.5)	56.2 (15) a	3.29 (1.1) b
Biosolids-22	0.24 (0.08)	5.97 (0.22)	54 (3.16) b	32 (3.20)	14 (0.957)	61.2 (4.6) a	5.89 (2.5) a
Biosolids-56	0.33 (0.19)	6.12 (0.16)	53 (2.65) b	35 (2.52)	12 (2.16)	60.6 (9.2) a	3.78 (0.88) b
Biosolids-112	0.34 (0.21)	6.21 (0.12)	53 (1.71) b	38 (6.40)	9.0 (5.56)	62.9 (4.3) a	4.29 (0.69) ab
Biosolids-224	0.36 (0.06)	6.13 (0.32)	54 (2.38) b	33 (2.06)	13 (2.63)	64.4 (8.9) a	5.32 (0.73) a
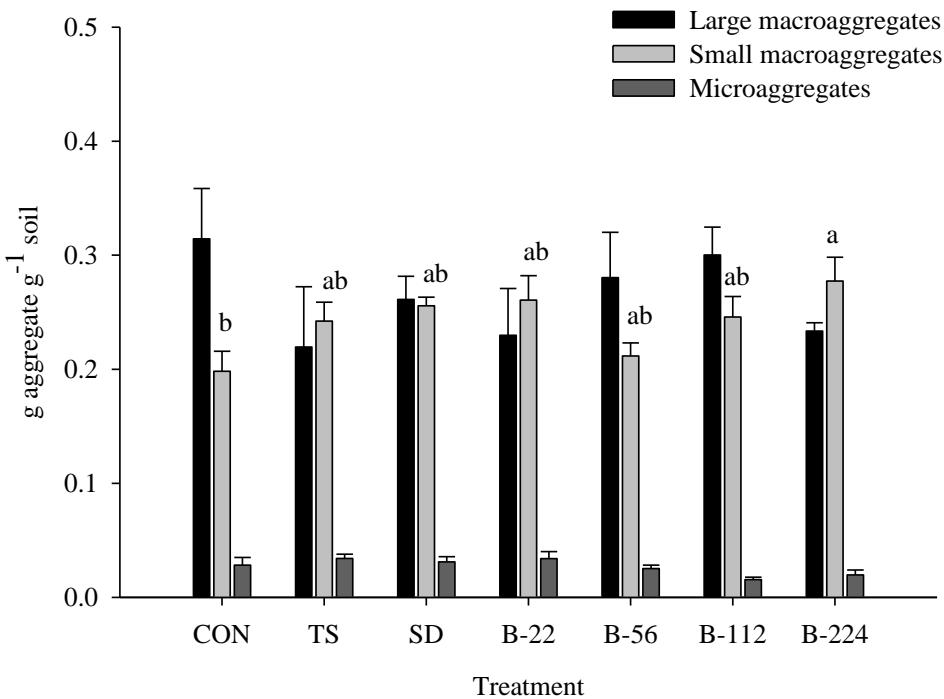
Figure 2. Soil aggregate size distributions for various amendments on the controlled overburden placement experiment located in southwestern Virginia. CON=control, TS=topsoil, SD=sawdust, B-22 = biosolids 22 Mg ha^{-1}, B-56 = biosolids 56 Mg ha^{-1}, B-112 = biosolids 112 Mg ha^{-1}, and B-224 = biosolids 224 Mg ha^{-1}. Error bars represent one standard error of the mean and letters indicate significant differences among treatments for each aggregate size class. Size fractions without letters indicate no differences among treatments (P<0.05). n=4.

Aggregate Carbon and Nitrogen

Large macroaggregate C and N concentrations were similar across treatments (P = 0.132, 0.118, respectively; Figs. 3 and 4). Small macroaggregate and microaggregate C and N concentrations were generally higher in the biosolids treatments (B-22, B-56, B-112, B-224) compared to the CON, TS and SD treatments. The B-22 and B-224 treatments were significantly higher in C than other treatments for both small macroaggregates and microaggregates, while values were more similar for N concentrations in these aggregate size classes. In general, biosolids treatments were higher in total aggregate associated C (as calculated by the sum of all aggregate C concentrations) than the CON, SD and TS treatments. Biosolids treatment B-22 contained 127.3 g total C kg^{-1} sand free aggregate, B-56 concentrations were 101.7 g total C kg^{-1}

sand free aggregate, B-112 contained 96.40 g total C kg^{-1} sand free aggregate and B-224 contained 118.3 g total C kg^{-1} sand free aggregate. The TS treatment C concentration was 45% lower than the B-22 treatment and the lowest concentration of all treatments (71.23 g total C kg^{-1} sand free aggregate). The CON treatment (75.70 g total C kg^{-1} sand free aggregate) and the SD treatment (90.94 g total C kg^{-1} sand free aggregate) were both significantly lower than all biosolids treatments (P=0.008). Similar trends were observed for N concentrations; where, biosolids treatments were significantly higher than other treatments (ranged from 6.67 to 7.98 g total N kg^{-1} sand free aggregate; P=0.004). The CON treatment C concentrations were the lowest (4.50 g total C kg^{-1} sand free aggregate), followed by the TS treatment (4.51 g total C kg^{-1} sand free aggregate) and SD treatment (5.43 g total C kg^{-1} sand free aggregate). Clearly, the biosolids treatments produced higher total aggregate C and N concentrations relative to other treatments.
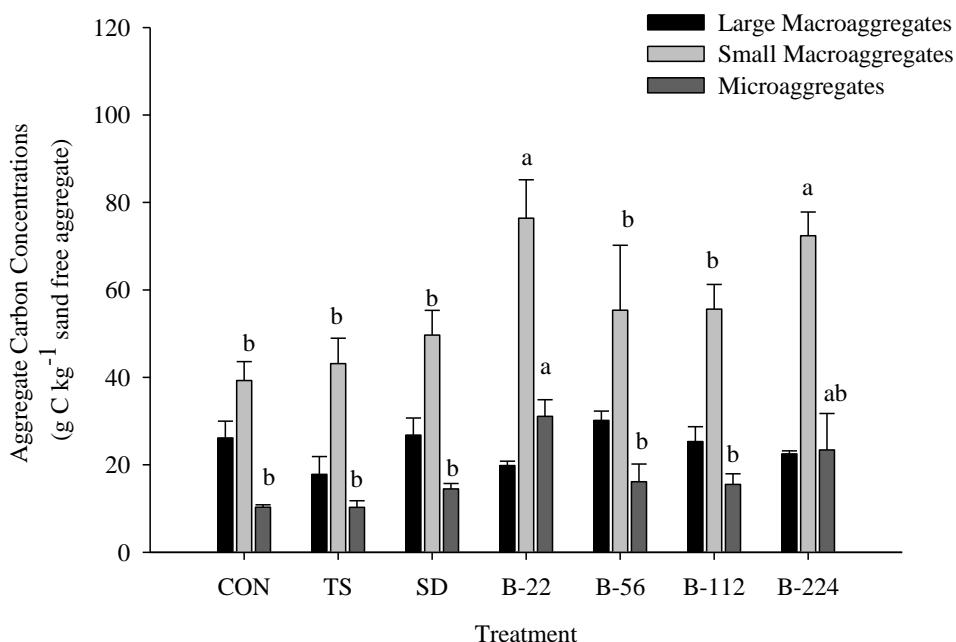

Figure 3. Aggregate associated carbon for various amendments on the controlled overburden placement experiment located in southwestern Virginia. CON=control, TS=topsoil, SD=sawdust, B-22 = biosolids 22 Mg ha^{-1}, B-56 = biosolids 56 Mg ha^{-1}, B-112 = biosolids 112 Mg ha^{-1}, and B-224 = biosolids 224 Mg ha^{-1}. Error bars represent one standard error of the mean and letters indicate significant differences among treatments (P<0.05). n=4.
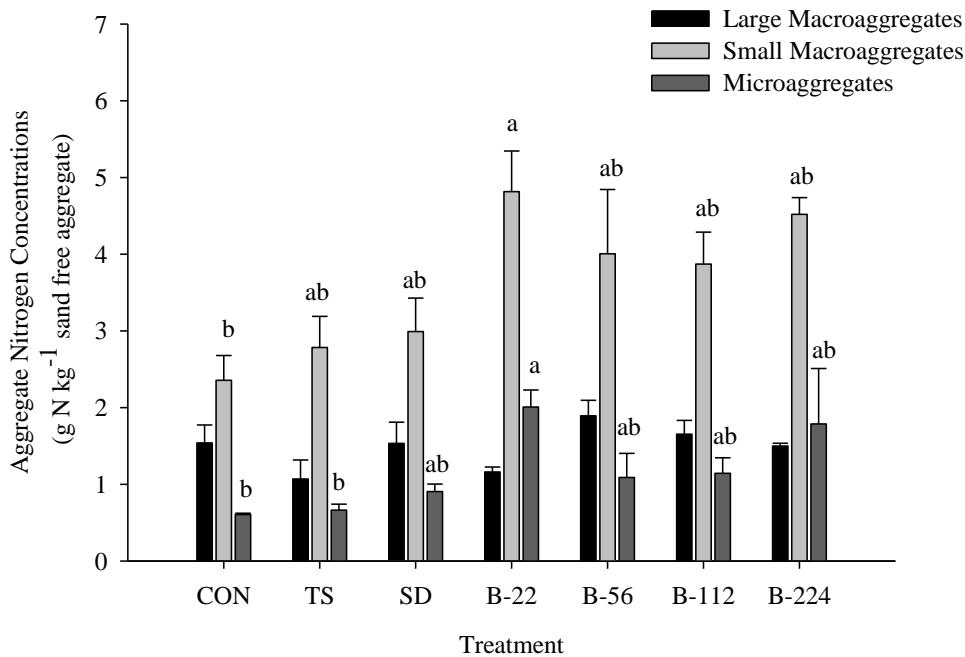
Figure 4.   Aggregate associated nitrogen for various amendments on the controlled overburden placement experiment located in southwestern Virginia. CON=control, TS=topsoil, SD=sawdust, B-22 = biosolids 22 Mg ha^{-1}, B-56 = biosolids 56 Mg ha^{-1}, B-112 = biosolids 112 Mg ha^{-1}, and B-224 = biosolids 224 Mg ha^{-1}. Error bars represent one standard error of the mean and letters indicate significant differences among treatments (P<0.05). n=4.

Organic Matter Fraction Carbon and Nitrogen

Free LF C concentration (considered available) was significantly higher in the B-56 treatment compared to B-112, B-224 and CON treatments (P=0.074 Fig. 5) and was significantly higher in available N compared to all other treatments (P=0.099; Fig. 6). There appears to be a biosolids loading rate response in the available C and N concentrations that was not observed in the total aggregate C and N concentrations. Concentrations increase between the B-22 and B-56 treatments and then decline to the B-112 and B-224 treatments. Aggregate protected C (iPOM) concentrations were similar across all treatments, while iPOM N values were significantly higher in the B-22, B-112 and B-224 treatments relative to B-56, CON, SD and TS treatments (P=0.144). The silt+clay fraction, which contains chemically bound OM, showed similar trends; with the highest C concentrations observed in the biosolids treatments (P=0.111) and highest N concentrations on the B-224 and B-112 treatments relative to all other treatments (P=0.021).
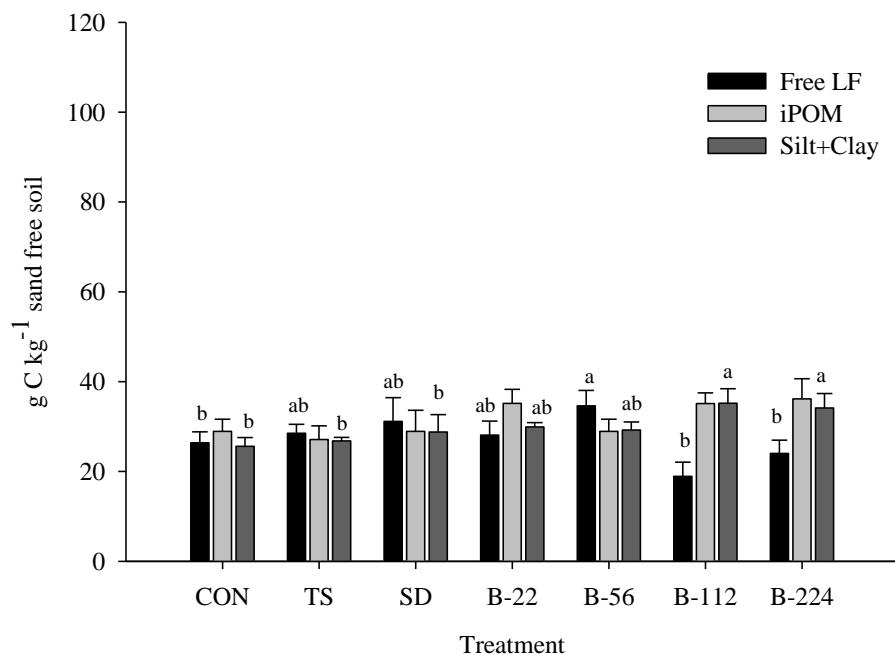
Figure 5. Carbon concentrations for whole soil fractions (inter-, intra-aggregate and organo-mineral) on a sand free basis various amendments on the controlled overburden placement experiment located in southwestern Virginia. Free LF=inter-aggregate, iPOM=intra-aggregate, and Silt+Clay= organo-mineral fraction. C=control, TS=topsoil, SD=sawdust, B-22 = biosolids 22 Mg ha^{-1}, B-56 = biosolids 56 Mg ha^{-1}, B-112 = biosolids 112 Mg ha^{-1}, and B-224 = biosolids 224 Mg ha^{-1}. Error bars represent one standard error of the mean and letters indicate significant differences among treatments (P<0.10). n=4.
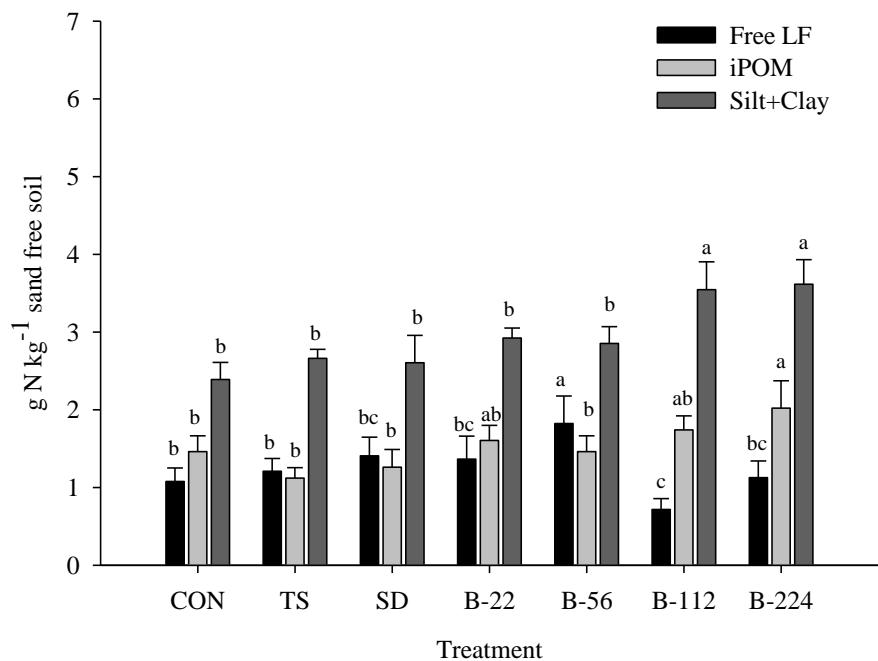
Figure 6. Nitrogen concentrations for whole soil fractions (inter-, intra-aggregate and organo-mineral) on a sand free basis various amendments on the controlled overburden placement experiment located in southwestern Virginia. Free LF=inter-aggregate, iPOM=intra-aggregate, and Silt+Clay= organo-mineral fraction. C=control, TS=topsoil, SD=sawdust, B-22 = biosolids 22 Mg ha^{-1}, B-56 = biosolids 56 Mg ha^{-1}, B-112 = biosolids 112 Mg ha^{-1}, and B-224 = biosolids 224 Mg ha^{-1}. Error bars represent one standard error of the mean and letters indicate significant differences among treatments (P<0.10). n=4.

Microbial Biomass Carbon

There were no differences observed for MBC among treatments; however, a biosolids loading rate response similar to that observed in the available C and N (Free LF) concentrations (Figs. 5 and 6) was observed for MBC (Fig. 7).
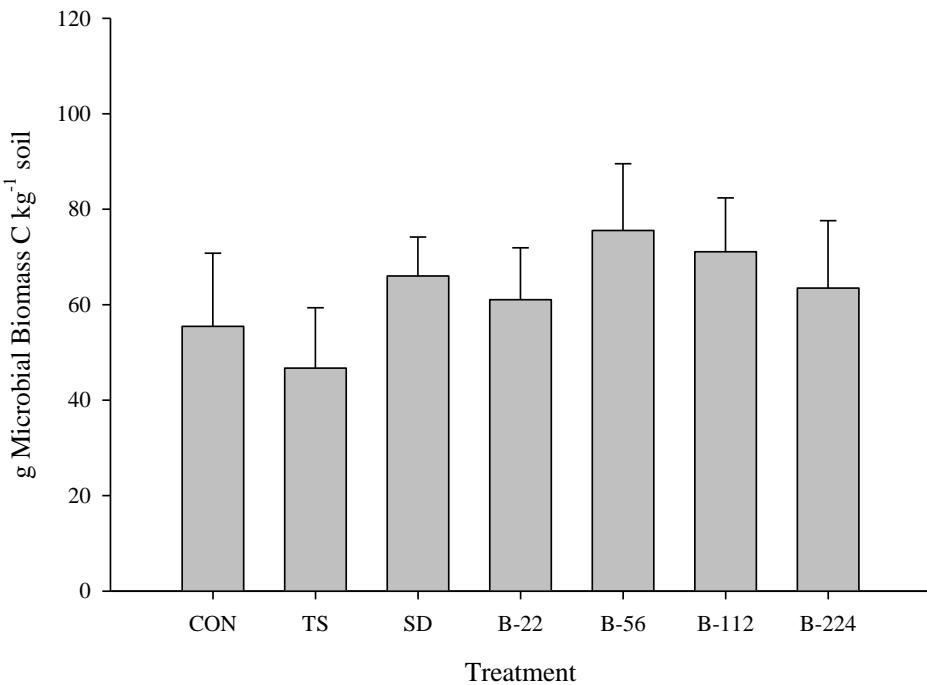
Figure 7. Microbial biomass carbon for various amendments on the controlled overburden placement experiment located in southwestern Virginia. C=control, TS=topsoil, SD=sawdust, B-22 = biosolids 22 Mg ha^{-1}, B-56 = biosolids 56 Mg ha^{-1}, B-112 = biosolids 112 Mg ha^{-1}, and B-224 = biosolids 224 Mg ha^{-1}. Error bars represent one standard error of the mean and letters indicate there were no significant differences observed among treatments (P<0.10). n=4.

Discussion

Soil development from mine spoils provides a unique opportunity to expand upon existing knowledge of aggregate formation and stabilization, OM accumulation and distribution and microbial biomass because of the magnitude of disturbance essentially creating a "time zero" scenario. Mining disturbances often result in a 50-70% reduction in soil OM occurring instantly (Shrestha et al., 2008; Wick et al., 2009a,b, c). This level of impact is even greater (>90%) in the Appalachian mining region where crushed rock is typically returned to a reclaimed area and stabilized with vegetation without topsoil salvage and return. Soil development then depends highly upon the replaced rock mixes, vegetation types, soil amendments, geomorphology and hydrology. Agricultural soils, where a majority of soil aggregate and OM research is conducted (Six et al., 1998; Jastrow et al., 1998), has been the focal point for existing theories on aggregation, aggregate hierarchy and OM dynamics. However, agricultural practices result in a

lower level of impact to soil aggregation and a more gradual decrease in the soil OM pool over a longer period of time (Shrestha et al., 2008). For example, a 60% reduction in soil OM might occur over a period of 60 years, averaging only a 10% loss per decade (Shrestha et al., 2008). This is vastly different from the instantaneous losses as a result of mining and we might expect aggregate and OM dynamics to be very different in reclaimed vs. agricultural soils. Both areas of study (mining and agricultural) provide opportunities to: (1) improve upon existing aggregate and OM theory, and (2) better understand modifications of soil management practices and utilization of soil amendments (typically in the form of mulches or fertilizer). Throughout the discussion, we will first interpret aggregation, OM and MBC in the soil development scenario (using primarily the CON plots) and relate it to existing aggregate theory followed by a comparison among soil amendments.

Aggregation

Close to 50% of the total soil was aggregated after 27 years of soil development, with 95% of total aggregation consisting of large and small macroaggregates. Although this does not approach the level of aggregation observed in early- and mid-successional abandoned agricultural fields (75-90% total aggregation; Grandy and Robertson, 2007), it was still impressive considering the aggregates formed and stabilized from weathering spoil material with very low clay contents (<20%). Clearly for soils in this region, aggregate formation after 27 years approached that of an undisturbed soil (typically observed with high macroaggregation and low microaggregation and free silt and clay). Aggregate theory suggests and has proven that microaggregates form and persist on the inside of macroaggregates (Oades and Waters, 1991). We can assume (without measurement) that the low "free" microaggregate weights indicated that a large portion of microaggregates were being formed and stabilized on the interior of the macroaggregates enhancing physical protection of OM. These microaggregates will eventually be released into the soil matrix as macroaggregates turnover (Six et al., 1998). Macroaggregate and associated C turnover time has been estimated to be approximately 40 years (Six et al., 2002), so we can assume that these 27 year-old soils are close to their height of aggregation. In the next decade, we might expect the macroaggregates to start turning over while at the same time re-forming to create a more even distribution of aggregate size classes.

Aggregate size distributions were similar among soil treatments, again with high macroggregation and minimal microaggregation. As for the benefits of soil aggregation on

physical properties (i.e. pore size distributions for air and water movement) all treatments would have similar effects after 27 years of soil development. The similarity among biosolids application rates, SD and TS treatments contributes to our current understanding of the influence of organic amendments on soil aggregation. Previous studies on the influence of biosolids applications on soil aggregate structure have indicated that there is a high initial aggregate formation following a single application of biosolids followed by lower aggregation (Metzger et al., 1987; Malik and Scullion, 1998). In this case, where there was a single application at a range of rates, the effects of the application on aggregation diminished after 27 years. Bendfeldt et al. (2001) measured aggregate stability on these same sites in 1998 (after 16 years of soil development) and found significantly higher aggregation on the B-22 and B-112 treatments compared to TS and similar aggregation among all biosolids treatments and the SD treatment. However, aggregates isolated by Benfeldt et al. (2001) ranged in size from 210 to 5000 μm, where in this study aggregates between 53 and 8000 μm were measured. Possibly, the initial stimulation of aggregation occurred within the first decade after plot establishment and treatment and was diminishing by 1998 during the Benfeldt et al (2001) study. More interestingly, the CON treatment achieved similar aggregation as the plots receiving surface amendments. Additions of OM (litter and roots) and C substrates (root exudates) to the soil, as well as the stimulation of microbial communities in the rhizosphere (as demonstrated by the CON treatment) were just as effective on aggregate formation and stabilization after 27 years as the addition of organic amendments. Overall, aggregation on the Controlled Overburden Placement experiment indicated similar levels of aggregate formation and stabilization during soil development regardless of treatment after 27 years.

Organic Matter

Organic matter is important for soil structure and function and is also the primary mechanism for terrestrial C storage (in addition to plant roots and biological soil properties). Carbon concentrations in the plots ranged from 55.02 to 64.42 g C kg^{-1} soil for most treatments (with exception of the TS treatment) and CON plots. In surrounding undisturbed soils, total soil C concentrations have been estimated to be 42.90 g C kg^{-1} soil (Orndorff, unpublished data). The undisturbed soil has lower total C concentrations, supporting previous indications of the potential for C storage in reclaimed mine soils (Fettweis et al., 2005; Shukla and Lal, 2005; Ussiri et al., 2006; Jacinthe et al., 2008; Wick et al., 2009a,b). It is important to realize that reclaimed soils

are often contaminated with coal dust, so reclaimed soil C concentrations could be inflated relative to undisturbed soils. Regardless, higher C concentrations in the reclaimed soils compared to undisturbed areas leads to questions of where the C is located in the soil. For instance, it is important to know if the accumulated C is physically protected, chemically bound to fine soil particles or is available for decomposition and release from the soil to the atmosphere as CO_2.

By isolating soil aggregates and OM fractions in soils, an estimate of available, protected and chemically bound C concentrations can be obtained. The total aggregate associated C concentration was 75.70 g C kg^{-1} sand free aggregate in the CON soils. Being aggregate associated, means that this C is both protected within aggregates and is free on the outside of aggregates for that size class. Based on theory, C concentrations should be higher in macroaggregates than microaggregates due to the less decomposed nature and higher C:N ratio of plant material protected by macroaggregates (Jastrow and Miller, 1998). This theory was supported when comparing the C and N concentrations across aggregate size classes in the CON soils; however the C:N ratios did not reflect a decomposition of plant material with decreasing aggregate size class. All three aggregate size classes (large and small macroaggregates and microaggregates) in the CON treatment had a 17:1 C:N ratio. Typically, the C:N ratio should decrease with aggregate size class (from ~20:1 in large macroaggregates to ~10:1 in microaggregates) and has been proven to do so in previous agricultural studies (Puget et al., 1995; Angers and Carter, 1996). The transfer of newly added OM to reclaimed soils among soil pools could have occurred at a faster rate between aggregate size classes in developing soils compared to the transfer observed in agricultural soils. This might explain the general ability of reclaimed soils to surpass the "threshold" of undisturbed soil C storage (Stewart et al., 2007). A specific threshold has been identified for various undisturbed soils by Six et al. (2002) suggesting that soils further from saturation (or their inherent threshold; i.e. reclaimed soils) are more efficient at accumulating C than an undisturbed soil. A soil closer to its specific threshold will accumulate C at a slower rate and be less efficient than a recently disturbed soil (Hassink and Whitmore, 1997; Chung et al., 2010). Therefore, C storage efforts associated with reducing atmospheric CO_2 contributions from soils should be directed at recently disturbed soils with a high capacity to accumulate C (Stewart et al., 2007) when and where possible.

Total aggregate C and N concentrations were significantly higher on the B-22, B-56 and B-224 treatments compared to other treatments. Interestingly, C concentrations were lower in the B-112 treatment relative to the other biosolids treatments. The TS treatment was consistently lower in total aggregate C and N compared to other treatments, despite inherent higher levels of total N in forest soils compared to reclaimed soils in this region (Li and Daniels, 1994). Biosolids or sawdust amendments were more conducive to C and N accumulation in general than the topsoil amendment. Aggregate C and N concentrations for each size class across soil treatments were similar, again with a majority of C and N in the small macroaggregate size fraction. As with the CON soils, the C:N ratios were similar across all aggregate size classes for all treatments indicating a quick transfer of newly added plant material among aggregate size classes. The N signature of the biosolids application was still evident in the small macro- and microaggregate fractions for these treatments 27 years after application.

When the whole soil was broken down into individual components (Free LF; iPOM and Silt+Clay), C concentrations were similar among each fraction for the CON plots. This is an "ideal" situation, where there are equal proportions of available C for quick utilization, physically protected for slow release and chemically bound for long-term storage. In a recently disturbed mine soil, a high portion of C is expected to be in the Free LF, followed by the iPOM and then Silt+Clay pool (Wick et al., 2009a,b). Free LF C values in the CON treatment were much lower than concentrations observed in a nearby undisturbed soil (45.2 g LF C kg^{-1} sand free soil; Wick et al., 2009c); however, iPOM concentrations in the CON soils were three times that of an undisturbed soil (11.6 g iPOM C kg^{-1} sand free soil). Perhaps this finding further supports the rapid transfer of OM into protected pools or mineralization of newly added OM in the reclaimed soils concept presented in this study. Nitrogen shows an interesting trend with higher concentrations in the organo-mineral Silt+Clay fraction. Both C and N in this fraction are highly decomposed and have been metabolized repeatedly by microbes (Golchin et al., 1994). We might infer that N was more rapidly cycled through the developing soil pools than C due to the N poor nature of crushed rock (Li and Daniels, 1994). The undisturbed soil N concentrations in the iPOM pool were much lower (0.458 g iPOM N kg^{-1} sand free soil; Wick et al., 2009c) than that observed in the CON soils (1.46 g iPOM N kg^{-1} sand free soil) while Free LF and Silt+Clay pools were similar. This again might be an indication of a rapid transfer between the active and the aggregate protected pools.

Carbon and N trends were similar for the different treatments; however, there was a slight biosolids loading rate effect on C and N pools. Carbon and N in the Free LF pool showed an increase between the B-22 and B-56 treatments, followed by a decline to the B-112 and B-224 treatments. This was also evident in the MBC results as microbial growth is dependent upon available C and N. Applying biosolids at a rate of 56 Mg ha^{-1}, proved to be the ideal rate for C and N availability.

Conclusions

Examination of soil development from mine spoil and the effects of soil amendments on soil development are crucial for our ability to improve our knowledge of aggregate dynamics, advance management practices and recognize beneficial soil amendments for both short- and long-term reclamation goals. First, reclaimed coal mine soils typically followed aggregate formation and OM accumulation theory based on numerous studies in agricultural and undisturbed soils. The C:N ratios for the aggregate size classes showed an accelerated transfer of plant material among aggregates compared to rates observed in agricultural soils. This was well supported by the C threshold theory and models recently developed, which state that C poor soils (such as reclaimed soils) accumulate C more quickly and efficiently than C saturated soils (undisturbed soils). Rapid accumulation in reclaimed mine soils resulted in C concentrations higher than observed in surrounding undisturbed soils and helps us realize the full potential of reclaimed soils for C storage. As for management recommendations, the benefits of biosolids applications are probably realized in <10 years; however, the N signature associated with biosolids applications was still apparent in the organo-mineral pool after 27 years. Remarkably rapid soil development with respect to aggregation and OM accumulation has been occurring on these mine spoils leading to fully functioning reclaimed ecosystems.

Acknowledgements

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