MICROBIAL BIOMASS IN RECLAIMED SOILS FOLLOWING COAL MINING IN VIRGINIA[1]

H.G. Clayton[2], A.F. Wick, and W.L. Daniels

Abstract: It is well known that soil microbial communities reestablish following disturbance, but limited research has been done on how long this takes in eastern reclaimed coal mine soils. The objective of this study was to track the development of total microbial biomass in reclaimed mine soils following coal mining in southwestern Virginia. A chronosequence of sites was established based on locally documented shifts in vegetation species with succession (0-2, 5-7, 18-20, and 38-42 yr old) on reclaimed sites with undisturbed adjacent sites used as a reference. The 0-2 yr old sites were covered with annual and perennial grasses and forbs, the 5-7 yr sites by thick stands of *Lespedeza cuneata*, the 18-20 yr sites predominately with *Festuca arundinaceae* and patches of deciduous trees, and the 38-42 yr old sites with a mix of planted conifers and native Appalachian hardwoods with a grass understory. Undisturbed sites predominately supported mixed Appalachian hardwoods with a mixed forb/grass understory. Four samples were taken from each site age (3 replicates) at the 0-5 cm depth. Chloroform-fumigation-extraction was used to determine total organic carbon from lysed microbial cells as a proxy for microbial biomass. Microbial biomass carbon (MBC) did not vary significantly among any of the site ages sampled; however, a general trend of increasing MBC from a low level in the more recently reclaimed sites (131-138 g microbial C kg^{-1} soil) to a higher level of MBC in the 16-20 and 38-42 yr sites (280 and 244 g microbial C kg^{-1} soil, respectively) was observed. This indicated a recovery of soil microbial communities with time; possibly well within the 5-year vegetation liability release period mandated by the Surface Mining Control and Reclamation Act (SMCRA).

Additional Key Words: Succession, chronosequence, Appalachian hardwoods, soil quality.

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Introduction

It is well known that soil microbial communities rapidly reestablish following disturbances (Shillam et al., 2008, Baldrian et al., 2008), but little research has been done on the status and health of microbial communities following coal mining in the eastern United States. Studies conducted on microbial shifts in reclaimed mine soils with time since disturbance show a significant reduction in microbial communities following mining followed by a general increase in microbial biomass and community diversity through time (Baldrian et al., 2008, Banning et al., 2008). Determining recovery times of microbial biomass in reclaimed systems is important because microbial associations with organic matter and nutrient cycling are critical for ecosystem function and availability of essential plant nutrients (Vogel, 1987), particularly nitrogen (N) and phosphorous (P).

Microbial communities play an important role in soil productivity by breaking down organic matter into inorganic forms of nutrients available for plant uptake (Paul & Clark, 1996). Soil aggregation, which is also sensitive to disturbance, provides a diverse habitat for microorganisms and physically protects organic matter from microbial decomposition. In addition to providing nutrients for plants in the traditional bottom-up approach of studying soil microorganisms, the quality of plant inputs affects the accumulation of microbial biomass carbon (MBC; Vogel, 1987; Ehrenfeld et al., 2005). A spike in the amount of available soil carbon (that not physically protected by aggregates, chemically bound to fine soil particles, or chemically complex and resistant to decomposition) leads to a positive response in MBC. Significant N inputs via plant or fertilizer inputs produce a similar positive response of MBC. These responses have been noted across different landscapes and vegetation types (Wardle, 2002). Although the plant communities and plant inputs varied due to naturally occurring succession in this study, we assumed similar MBC responses to these changes as the sites aged.

Microbial diversity and MBC increase over time following disturbances. One study found age of soils (7,500 years *vs* 300 years old) was not a factor controlling the resiliency of the soils to disturbance; although the older soils did have higher diversity in community structure (Shillam et al., 2008). This study demonstrated that MBC increases over time following a disturbance. Studies have also been conducted on MBC and soil organic carbon (SOC) on reclaimed mine soils in Wyoming. Anderson et al. (2008) reported significantly higher MBC in undisturbed soils than in a chronosequence of reclaimed soils 11, 14 19, and 26 years after surface coal mining, indicating microbial recovery after 20+ years. Another study, conducted near Perth in Western Australia, found soil microbial biomass increased quickly with succession following disturbance and then decreased and leveled off due to limited availability of soil organic matter which was protected by newly formed soil aggregates (Banning et al., 2008). Obviously, microbial response to disturbance is regionally specific and there is a need to understand these dynamics in other mining regions such as the Appalachian coal basin.

The objective of this study was to determine the content of MBC in reclaimed mine soils of varying age following cross ridge coal mining in southwestern Virginia. We hypothesized that: (1) the undisturbed soils would have the highest MBC compared to the reclaimed soils and (2) the 0-2 yr age mine soils would have the lowest MBC relative to the other reclaimed site ages. We assumed that the intermediate age soils would fit into an increasing trend between the low and high MBC values.

Materials and Methods

Site Description

Sampling sites were chosen near the Powell River Project Education Center in Wise County, VA. Extensive research has been conducted in this area since 1988, primarily by Virginia Tech. Cross ridge mining in this area has created a classic rolling bench and backfill topography on which spoil was deposited. The mine soil parent materials were mostly sandstone and siltstones with traces of carbonates, but low in coal/carbonaceous materials. The mine soils in the study area were classified primarily as loamy, mixed, mesic Typic Udorthents by Haering et al. (2004). For Wise County, VA, the average temperature in January is 0°C and in July is 21°C. Average snowfall per year is 94 cm and average rainfall is 116.8 cm (Wise Co., 2006).

A chronosequence of sites was established to evaluate microbial recovery following mining. Sites were chosen with similar characteristics, such as slope and landscape position, but these sites cannot always be found given the variability associated with reclamation techniques through time. Three replicates of sites ranging in age from 0-2, 5-7, 16-20, and 38-42 yrs since reclamation, plus undisturbed sites were located based on availability of site ages (Fig. 1). All sites, except the oldest, were reclaimed by the same company (Red River Coal) and received similar reclamation treatments; however, the 16-20 yr sites received a single application of biosolids. We still felt that capturing soils under 16-20 yr old successional communities was important to this study. All sites were reclaimed with overburden derived topsoil substitutes without return of salvaged topsoil. However, the spoils at the older sites contained a higher proportion of significantly pre-weathered and oxidized near-surface overburden (Haering et al., 2004). The 0-2 yr old sites were covered with annual and perennial grasses and forbs, the 5-7 yr sites by thick stands of *Lespedeza cuneata*, the 16-20 yr sites predominately with *Festuca arundinaceae* mixed with invading forbs and patches of deciduous trees, and the 38-42 yr old sites with a mix of planted conifers and native Appalachian hardwoods with a grass understory. Undisturbed sites were a natural mixture of native deciduous trees and understory species. One of the 16-20 yr sites is currently grazed by cattle.

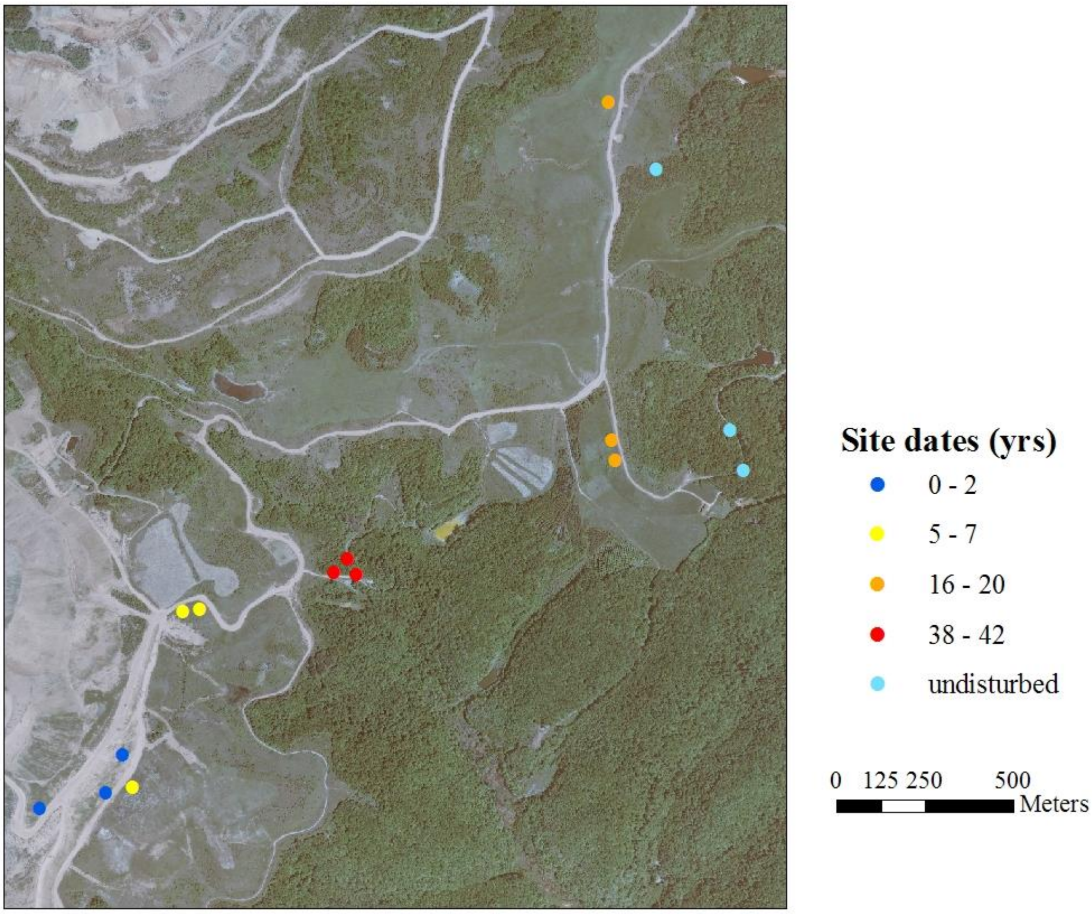

Figure 1. Locations for a chonosequence of sites located in the coal fields of southwestern Virginia.

Field Sampling

Sampling was conducted in August and November, 2008. First, the vegetation was clipped off the surface of the soil. The upper 5 cm of soil was collected with a trowel at four equidistant locations along a 4 meter transect. Samples were kept cool in the field to reduce any microbial activity and stored at 4˚C for no longer than three weeks until analyses were performed.

Sample Preparation and General Soil Analyses

An 8 mm sieve was used to separate coarse fragments from the sample and identifiable pieces of organic matter were taken out with forceps prior to MBC analysis. A subsample for each replicate was weighed at approximately 5 g, dried overnight at 105°C, and weighed again to determine gravimetric moisture content. A subsample of soil was dried and sieved to 2 mm for electrical conductivity (EC) and pH with a 1:1 soil:water mixture and particle size analyses. 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).

Chloroform-fumigation extraction

The modified chloroform fumigation extraction method used in this study was based on methods by Coleman et al. (2004) and von Luetzow et al. (2007). Briefly, 10 g of 8 mm sieved field-moist soil were transferred to 50-mL beakers. Samples were then fumigated for 24 h with chloroform under vacuum and left to ventilate under a fume hood for another 24 h. Samples were then transferred to 125-mL Erlenmeyer flasks and shaken for thirty minutes with 50 mL 0.25 M K_2SO_4. The supernatant was then poured through Whatman #42 filter papers folded into cones to filter by gravity. Solution samples were immediately analyzed with a Total Organic Carbon (TOC) Analyzer (Sievers 900, Boulder, CO). This process was repeated for both fumigated (F) and non-fumigated (NF) samples. Equation 1 was used to determine MBC in g C kg^{-1} soil.

$$MBC = \frac{TOC\ (F) - TOC\ (NF)}{Kc} \quad \textbf{(1)}$$

where Kc = 0.38 (von Luetzow, 2007)

During sample processing, certain soluble constituents became insoluble after filtering for carbon extraction in both fumigated and non-fumigated sets. To prevent the salts from precipitating, the K_2SO_4 extracting solution was lowered from 0.5 M to 0.25 M (Haney et al., 2001). All samples were field-moist when processed, and dry weight correction factors were applied to correct the microbial biomass data with gravimetric moisture content.

Statistical Analyses

Average values from each replicate were analyzed using one-way analysis of variance using SigmaPlot (SigmaPlot, 2008). Pearson’s correlation was used to relate MBC to other soil parameters (aggregation and organic matter) collected by Wick and Daniels (2009).

Significance was determined at P<0.05.

Results and Discussion

Soil EC was similar across site age (Table 1). The pH was higher in younger reclaimed soils because 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 pre-weathered overburden in the older mine soils (Haering et al., 2004). The 5-7 yr site had significantly higher clay and lower sand than the other reclaimed sites. The samples ranged from 5% to 55% water by weight at time of sampling.

Table 1. Electrical Conductivity (EC), pH, and soil texture for a chronosequence of sites in the coal fields of southwestern Virginia. Significant differences shown across reclaimed site age with lower case letters (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 (yrs)	**EC** ($dS\ m^{-1}$)	**pH**	**Texture**
0-2	0.36 (0.09) a	8.08 (0.10) a	Loam/Sandy Loam
5-7	0.57 (0.24) a	6.27 (1.1) ab	Silt Loam/Loam
16-20	0.58 (0.13) a	5.93 (0.49) ab	Sandy Loam/Loam
38-42	0.32 (0.03) a	5.38 (0.41) b	Loam
Undisturbed	0.44 (0.14)	5.75 (1.16)	Sandy Loam

There were no significant differences in MBC observed among any of the ages due to high variability within site replicates (P = 0.119; Fig. 2); however, there was a strong indication that MBC doubled with reclaimed site age from 130.8 g microbial C kg^{-1} soil in the 0-2 yr sites to 280.7 g microbial C kg^{-1} soil in the 16-20 yr sites. Cavigelli et al. (2005) found that soil microbial properties are highly variable with soil type, date of testing, and landscape elements. This could have been the cause for the high variability observed here.

Anderson et al. (2008) report that it usually takes 20 years or more for microbial biomass and diversity in disturbed soils to reach the levels of native soils in the semiarid West and soil MBC increased immediately post-disturbance in the Banning et al. (2008) study conducted in Western Australia. In our study, the 0-2 yr soils appeared to be similar the 5-7 age group followed by an increase to the 16-20 yr site. Banning et al. (2008) support this assumption by stating labile sources of carbon inputs are utilized quickly by microbial communities, resulting in a rapid increase in MBC. A study in the subtropical climate of northeastern Cuba observed similar microbial enzyme levels within six years after two species of trees were planted in reclaimed locations (Izquierdo et al., 2005). These findings also support our apparently quick recovery of MBC within 2 years of disturbance, which is well within the 5-year vegetation performance liability period dictated by SMCRA (1977).

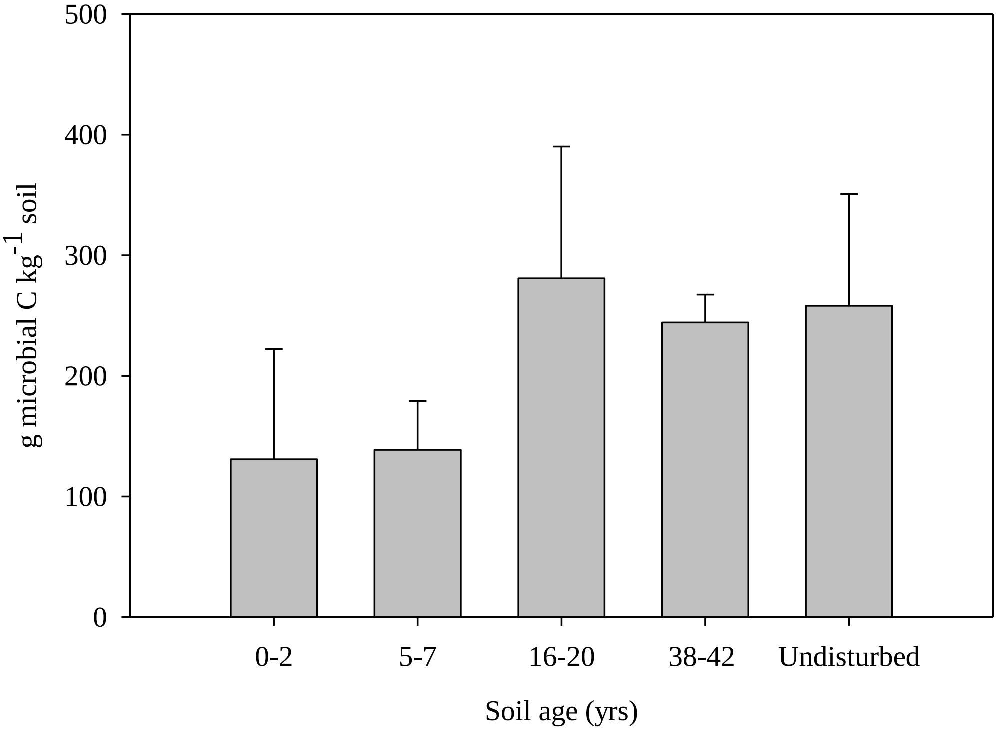

Figure 2. Microbial biomass carbon (g C kg^{-1} soil) values for the chronosequence studied. No differences (P = 0.120) were found, and it was concluded that after two years the microbial community had been restored to near undisturbed levels. Error bars represent one standard error of the mean.

Some interesting relationships were observed in collaboration with Wick and Daniels (2009) who sampled the same sites for organic matter and aggregate dynamics. Microbial biomass carbon was highly related to inter-aggregate particulate organic matter (or that not physically protected and available for microbial utilization; R^2 = 0.70, P = 0.01) and intra-aggregate particulate organic matter (that physically protected by soil aggregates; R^2 = 0.81, P = 0.001). The tight link between organic matter and MBC further indicates a "recovered" reclaimed ecosystem (Vogel, 1987).

We speculate soil heterogeneity, short recovery periods of microbial communities, and differences in soil moisture (Cavigelli et al., 2005) led to a recovery trend but also a lack of statistical significance in this study. In our study, all of the age groups had different vegetation types as a result of succession. For instance, the 38-42 yr sites were forested whereas the 0-2 yr sites had a thin covering of grasses and forbs. Soil heterogeneity can be influenced by different vegetation, even in areas located close to each other. For example, Mummey et al. (2002) found that shrubs in high wind areas served as windbreaks and trapped leaves around their base. This process locally increased levels of SOM and thus microbial and fungal markers were higher in those areas.

Identifying and collecting samples from 16-20 yr sites that were not treated with biosolids would create a better chronosequence and help with interpretation of our results. Additionally, continued work with the current samples pertaining to microbial activity (lab incubations) would be beneficial. Future general suggestions include creating a database for the reclaimed sites in southwestern Virginia, which currently does not exist. More detailed, documented information is vital to site selection as well as development of future studies.

Conclusions

The MBC of soils in a chronosequence of eastern Appalachian coal fields were not different among the age groups statistically, but appeared to increase with site age and reached a level similar to the undisturbed soil within 16-20 yrs. Our current findings support a 5-year bond release period for successful reclamation mandated by SMCRA (1977); however, more work is needed to decrease the variability among replicates within each site age and possibly a non-parametric statistical test should be used to better separate differences among site ages.

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