# Responses to Reviewer Comments
# "Soil biochemical properties after six years in amended brown and gray mine soils in West Virginia"

by C. Thomas et al.

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Reviewer #1

1. A reviewer asks if there was a surface organic layer on the areas where we sampled (page 679).

Reply: These are still very young soils since only 6 years have passed since reclamation and very little above ground cover exists (only 40% total cover at the most, Table 4), therefore, no organic layer has developed either as a surface litter horizon or a well-defined A horizon. There was no reason to sample the soil separately from the 0 to 15 cm in depth.

Therefore, I added a sentence

"No surface organic layer was present either as a litter layer or as an A horizon because insufficient time had passed for pedogenic processes to occur."

2. A reviewer asks about the effect of legumes on influencing nitrogen content of the mine soils (page 681).

Reply: It is true that several legumes were seeded as part of the hydroseeding mix, namely birdsfoot trefoil, Kobe lespedeza and Ladino clover. We did not show the cover composition data, but the legumes contributed a very small amount of the total herbaceous cover, which was only 40% or less of total cover. The grasses, particularly perennial ryegrass and orchardgrass, were the predominant herbaceous plants on these sites with sparse cover.

Therefore, I added two sentences:

"Perennial ryegrass (Lolium perenne) and orchardgrass (Dactylis glomeratus) were the predominant herbaceous species found accounting for more than 75% of the total herbaceous cover. Legumes in the seeding mix did not establish well and only made up a small part of the herbaceous cover."

While we did not measure the effect of possible nitrogen- fixation by these legumes and their contribution of N to the soil, due to the low amount of cover contributed by the legumes to the small amount of total cover, we think that the nitrogen effect is not due to nitrogen fixation, but much more likely due to the N fertilizer application.

I added the following sentences to address this issue:

"Nitrogen-fixing legumes were introduced by hydroseed application, which could have influenced the PMN:TN ratio. The effect of legumes on N cycling is unclear since the total N content of the mine soils with and without hydroseed was not different and the PMN on hydroseed plots was significantly greater in both mine soils. The herbaceous cover contributed by seeded legumes was very low compared to the seeded grasses, therefore it was assumed that the N differences were likely due to the earlier N fertilization."

3. The reviewer says that since MBC and PMN are significantly affected by hydroseeding, it makes no sense to discuss the overall mean of subplots on different substrates, and the values given in the text confuse with the data in table 7.

Reply: I agree and have eliminated two sentences on overall means for mine soils. Therefore, I have re-written the section emphasizing the hydroseed effects. And the reviewer is also correct that incorrect values were used in the text and didn't match the values in the tables. These have been corrected.

Microbial biomass carbon (MBC) was nearly identical between brown and gray mine soils (13.1 vs 13.0 mg $kg^{-1}$, Table 7). A similar result was found for PMN between brown and gray (0.58 vs 0.57 mg $kg^{-1}$) mine soils. Hydroseed application had a significant effect on MBC in brown mine soils and PMN in both mine soils (Table 7). MBC in hydroseed plots on brown mine soils had a mass of 17.5 mg $kg^{-1}$ compared to 8.7 mg $kg^{-1}$ in non-hydroseed plots. Mean PMN in hydroseed treatments was almost triple (0.81 mg $kg^{-1}$) that in non-hydroseed (0.35 mg $kg^{-1}$). MBC on our gray mine soils were similar varying between 12.8 and 13.3 mg $kg^{-1}$. However, PMN on gray hydroseed plots was more than three times greater at 0.92 mg $kg^{-1}$ compared to non-hydroseed plots having 0.22 mg $kg^{-1}$.

Reviewer #2

1. Reviewer wonders if title should be more explicit than the term "amended."

Reply: I agree, and therefore have changed the title to "Soil Biochemical Properties in Brown and Gray Mine Soils With and Without Hydroseeding"

2. Reviewer asks if a brief statement about the differences in these two materials is warranted.

Reply: I agree again, and therefore have added the following to the introduction.

"The brown sandstone substitute materials have a pH from 4.5 to 5.5 compared to the gray sandstone materials with a pH of 7.5 to 8.0 (Wilson-Kokes et al., 2013b). These low pH conditions are more conducive to tree growth, while the higher pH is better for many seeded grasses (Zipper et al., 2013). Both soils tend to contain high levels of rock fragments, which translates into poor water-holding capacity and poor nutrient relations in these topsoil substitutes (Haering et al., 2004).

3. Add soil texture and rock fragments.

Reply: I agree, and put in the following, "with soil textures of silt loam and sandy loam (15 to 25% rock fragments) ..."

4. Reviewer cautioned us against pseudo-replication.

Reply: We were careful to randomly place 13 transects across the site so that we would sample the plots in a way that would reflect the conditions represented on the site.

5. Lack of baseline data or even a reference point is a drawback.

Reply: Yes, I agree that biochemical measurements over time would have been the best way to conduct this study. Nevertheless, the fact that we have untreated brown and gray mine soils, and these same mine soils treated with and without hydroseeding is the data comparison we had for this study.

6. Coal that has been weathered can be thermally oxidized along with soil organic matter, underestimating Coal-C and overestimating soil organic C.

Reply: We agree that this is a concern. The temperatures selected for burning our samples (950 and 350) are well-known temperatures that are used to operationally separate carbon into these different fractions. It is also well-known that these temperatures are not strict boundaries where no coal carbon burns at 340 degree or less, and that all soil organic carbon is burned at 340 degrees. Because of the carbonate and coal fractions, we attempted to eliminate those fractions and not consider them as organic C in our calculations. It is also true that the weathered soils may have altered the burning temperatures for coal. Nevertheless, our study is unique in that we removed the interference of carbonates and coal C for our soil organic carbon interpretations.

7. If followed procedure closely, no need to give detailed steps if cited procedure. Just when modifications to procedure were done.

Reply: We feel that this description is necessary for the reader to be clear on our methods. Therefore, we don't want to remove it.

8. What type of mean separation test was run?

Reply: This was an oversight and we have added another sentence,

"Treatment means when found significantly different by analysis of variance were separated by Tukey's Honest Significant Difference test at the $p < 0.05$ level."

9. What about coal N? Coal-C was discussed, but not Coal-N?

Reply: We measured coal N and it was consistently about 5% of the Total N. Therefore, we didn't include it in the table, but did include a statement in the text "and coal N was about 5% of the total N (data not shown)."

10. Brown mine soil was significantly lower when compared to gray and brown mine soil with hydro seeding. Pooling of results is not appropriate here. Perhaps, the brown mine soil with no hydroseeding resulted in the lowest MBC because of having lower pH and rhizosphere exudates.

Reply: The reviewer is correct and I have already changed this section with the first reviewer's comment.

11. More discussion on how hydroseeding (specifically, species populations) affects C and N cycling is needed, since they are coupled cycles.

Reply: We didn't elucidate or expand the discussion on the effects of particular species on C and N cycling (like legumes) because the legumes were a very small part of the total low herbaceous cover. I think the species are less important than the original fertilizer that was applied to enhance growth of herbaceous plants.

12. Actual plant species populations in 2012 are needed.

Reply: We did not measure the percent cover of each species in our quadrats, but again, in hindsight, it would have been helpful.

13. Define "fines", assuming it's less than 2 mm sieved soil.

Reply: Agree, so added a footnote in Table 5.

[€]Material that passed a 2 mm sieve.

# SOIL BIOCHEMICAL PROPERTIES IN ~~AFTER SIX YEARS IN AMENDED~~ BROWN AND GRAY MINE SOILS WITH AND WITHOUT HYDROSEEDING ~~IN WEST VIRGINIA~~

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**Abstract.** Surface coal mining in the eastern USA disturbs hundreds of hectares of land every year and removes valuable and ecologically diverse eastern deciduous forests. Reclamation involves restoring the landscape to approximate original contour, replacing the topsoil, and revegetating the site with trees and herbaceous species to a designated post-mining land use. Re-establishing an ecosystem of ecological and economic value as well as restoring soil quality on disturbed sites are the goals of land reclamation, and microbial properties of mine soils can be indicators of restoration success. Reforestation plots were constructed in 2007 using weathered brown sandstone or unweathered gray sandstone as topsoil substitutes to evaluate tree growth and soil properties at Arch Coal's Birch River Mine in West Virginia, USA. All plots were planted with 12 hardwood tree species and subplots were hydroseeded with an herbaceous seed mix and fertilizer. After six years, average tree volume index was nearly ten times greater for trees grown in brown (3853 $cm^3$) compared to gray mine soils (407 $cm^3$). Average pH of brown mine soils increased from 4.7 to 5.0, while gray mine soils declined from 7.9 to 7.0. Hydroseeding doubled tree volume index and ground cover on both mine soils. Hydroseeding doubled microbial biomass carbon (MBC) on brown mine soils (8.7 vs 17.5 mg $kg^{-1}$), but showed no effect on gray (13.3 vs 12.8 mg $kg^{-1}$). Hydroseeding also increased the ratio of MBC to soil organic C in both soils and more than tripled the ratio for potentially mineralizable nitrogen (PMN) to total N. Brown mine soils were a better growth medium than gray mine soils and hydroseeding was an important component of reclamation due to improved biochemical properties and microbial activity in mine soils.

**Abbreviations:** EC, Electrical Conductivity; MBC, Microbial Biomass Carbon; PMN, Potentially Mineralizable Nitrogen

**Additional Key Words:** biochemical ratios, fertilization, hydroseeding, land reclamation, microbial biomass carbon, potentially mineralizable nitrogen, reforestation, revegetation, soil microbiology

## 1 Introduction

Surface mining in the eastern USA disturbs hundreds of hectares of land by removing ecologically diverse eastern deciduous forests, resulting in the disruption and degradation of underlying soil resources. In order to restore soil function and stability to these ecosystems (Brevik et al., 2015), a sufficient medium for plant growth must be re-established through reclamation. The most ideal material to place on the surface is the pre-existing soil material (Skousen et al., 2011; Zipper et al., 2013). Currently, brown and gray sandstones often are employed as substitutes for topsoil (Emerson et al., 2009; Skousen et al., 2011~~; Wilson-Kokes et al., 2013a and b; Zipper et al., 2013~~).~~,~~ The brown sandstone substitute materials have a pH from 4.5 to 5.5 compared to the gray sandstone materials with a pH of 7.5 to 8.0 (Wilson-Kokes et al., 2013a and b). These low pH conditions in brown mine soil are more conducive to tree growth, while the higher pH of gray mine soils is better for many seeded grasses (Zipper et al., 2013). Both mine soils tend to contain high levels of rock fragments, which translates into poor water-holding capacity and poor nutrient relations in these topsoil substitutes (Haering et al., 2004). ~~h~~However the temporal dynamics of the~~ir~~ physical, chemical, and biochemical properties of topsoil substitutes following reclamation are not sufficiently understood.

Microorganisms are known to play an important role in re-establishing soil organic matter content and restoring ecosystem services following surface mining or other land disturbances (Anderson et al., 2008; Machulla et al. 2005; Haney et al., 2008; Ingram et al. 2005; Zipper et al. 2011). However, their biochemical activities can be slow to re-establish (Chodak, 2009) and may take several decades to reach stable conditions normally found in native soils (Chatterjee et al., 2009; Insam and Domsch, 1988). Soil carbon amendments stimulate microbiological activity (Bendfeldt, 2001; Elkins et al., 1984; Lindemann et al., 1984) as can the establishment of herbaceous plants using the additions of fertilizers and lime (Chaudhuri et al., 2015). However as easily decomposable organic matter is rapidly consumed, an overall decline of microbial activity results if no further additions of external nutrients and organic matter are applied because a reservoir of soil organic matter is lacking in newly created mine soils (Stephens et al., 2001; Stroo and Jencks, 1982). Compaction of soil materials and planting of competitive forage species have been shown to arrest the re-colonization of native hardwood tree species and slow natural succession on these reclaimed sites (Franklin et al., 2012; Groninger et al., 2007), which also tends to diminish microbial diversity and activity. Hydroseeding to introduce lime, fertilizer, mulch, and seed is already a common practice in surface mine reclamation, and using lower rates of seed, fertilizer and lime, as well as using different herbaceous species aid the development of forests on these sites (Franklin et al., 2012; Showalter et al., 2010). Hydroseeding a non-competitive, tree-compatible herbaceous forage mix can still meet the requirements for soil stabilization and conditioning, and may also be useful for re-establishing microbial communities that aid in nutrient cycling (Zipper et al., 2011). The objective of this study was to determine tree growth and soil properties including microbial biomass carbon (MBC), and potentially mineralizable nitrogen (PMN) in brown and gray mine soils and to determine the influence of a hydroseed treatment on these properties.

## 2 Materials and Methods

Arch Coal's Birch River Operation is located near Cowen in Webster County, West Virginia, approximately 100 km northeast of Charleston (38°25'31" N 80°36'39" W). Coal from the Kittanning, Clarion, Stockton and Coalburg seams were mined at the site. Overburden was moved from above the seams by shovels, front end loaders, bulldozers, trucks, and dragline. The vegetative cover on pre-mining land was a mixed hardwood forest. Gilpin and Gilpin-Dekalb series (Typic Hapludults) with soil textures of silt loam and sandy loam and containing 15 to 25% rock fragments were the pre-existing soil types on the moderate to steep slopes of the region (Delp, 1998).

In January 2007, a 4.9-ha plot was created using two types of sandstone overburden. Half of the area was constructed with weathered brown sandstone, the other half with unweathered gray sandstone. Overburden materials were end-dumped into conjoining piles that were approximately 1.5-m deep throughout the plot. To limit compaction, a bulldozer made only one pass over the piles to strike off the tops, resulting in approximately 1.2-m depth of rough-graded material. In spring 2007, 12 species of tree seedlings were purchased from the West Virginia State Tree Nursery and planted on 2.4-m centers by Williams Forestry, a professional tree planting company, at a stocking rate of 1,680 trees per ha (Table 1). In the fall of 2008, both ends of the plot were hydroseeded with a seed mix of compatible herbaceous species at a rate of 36 kg $ha^{-1}$ (Table 2), and fertilized at a rate of 336 kg $ha^{-1}$ of 10-20-10 ($N$-$P_2O_5$-$K_2O$).

## 3 Sampling and Analyses

Survival and growth of trees as well as soil physical and chemical properties were measured from 2007 to 2012 as reported in Wilson-Kokes et al. (2013b). Tree growth was assessed by measuring height and stem diameter at 2.5 cm above ground, and a tree volume index was calculated as height x diameter$^2$. Percent ground cover from herbaceous plants, litter, and trees were also determined and reported as above.

Soil samples were collected from the top 15 cm at four randomly selected points along transects within each treatment combination in July of 2007 to 2012. No surface organic layer was present either as a litter layer or as an A horizon because insufficient time had passed for pedogenic processes to occur. Field soils were air dried and sieved through a 2-mm sieve to separate the fine soil fraction (<2 mm) from the coarse or rock fraction (≥2 mm). The fine soil fraction was used for all chemical and biochemical analysis.

Soil pH was measured in a 1:2 mixture of 5 grams of soil and 10 mL of DDI water with a Fisher Scientific Accumet pH meter model 915 (Thermo Fisher Scientific Inc., Pittsburgh, PA). Electrical conductivity (EC), was measured using a 1:2 mixture on a Mettler Toledo S230 EC Meter (Mettler-Toledo International Inc., Columbus, OH). Nutrients were determined using a Mehlich 1 extraction solution (0.05*M* HCl and 0.025*M* $H_2SO_4$), but are not reported here and results can be found in Wilson-Kokes et al., 2013b).

Biochemical measurements were made only on soil samples taken in 2012. Total carbon (TC) and nitrogen (TN) were measured with a Leco TruSpec CHN elemental analyzer (LECO Corp. St Joseph, MI). A 0.10-g sample of air-dried soil was weighed into foil cups and combusted at 950°C. Carbon fractions were also determined. Carbonate C was determined in soils by reaction with a 1*M* HCl solution as described by Ussiri and Lal (2008). For soil organic C, 1 g of soil was weighed into ceramic crucibles and thermally oxidized at 340°C for 3 hrs, after which samples were reweighed. Coal C was calculated as the difference between TC minus carbonate C and soil organic C.

Microbial biomass carbon (MBC) of the soil was determined using the chloroform fumigation extraction method from Brooks and Joergensen (2006). Two triplicate sets of 10-g (dry weight) samples of field moist soil where weighed into 125-mL glass serum bottles. One set of triplicate samples was designated as a control and did not undergo chloroform fumigation. Fumigated samples were exposed to 2 mL of amylene-stabilized chloroform. Airtight rubber stoppers were used to cap bottles prior to creation of a vacuum by pulling air from each bottle, followed by incubation for 24 hrs in the dark prior to extraction. Both fumigated and control samples were extracted with 25 mL of 0.5*M* $K_2SO_4$ on a reciprocating shaker for 60 min.

Samples were vacuum filtered through 0.45 μm Millipore filters yielding a soil free extract which was frozen until analyzed using a Sievers 5310C Total Organic Carbon Analyzer (GE Analytical Instruments. Boulder, CO). MBC was calculated as the difference between the control and fumigated samples divided by an extraction efficiency factor of 0.35.

Potentially Mineralizable Nitrogen (PMN) was determined using the anaerobic incubation method (Canali and Benedetti, 2006). Non-incubated control triplicate samples were created by weighing 16 grams (dry weight) of field moist soil and adding 40 mL of distilled deionized water into 250-mL glass Erlenmeyer flasks. Soil was extracted with 40 mL of 2*M* KCl for 60 min on a reciprocating shaker. Supernatant was filtered through 0.45 μm Millipore filters and frozen until further analysis. Anaerobically-incubated triplicate samples were created by weighing 16 grams (dry weight) of field moist soil into 50-mL polypropylene centrifuge tubes. Soil was suspended in 40 mL of distilled deionized water, the tubes stoppered, then incubated at 40°C for 7 days. Every 24 hrs during the incubation period, soil was re-suspended in solution. After the incubation period, the soil-water solution was transferred to a 250-mL glass Erlenmeyer flask. Centrifuge tubes were rinsed four times with 2*M* KCl into the same Erlenmeyer flasks. Samples were extracted, filtered and stored in a similar manner to control samples. A colorimetric method described by Mulvaney (1996) was used to determine the amount of nitrogen in the form of ammonium ($NH_4^+$) present in the filtered supernatants. The difference between non-incubated and incubated samples was calculated and recorded as the PMN.

Total C, C fractions (soil organic C, carbonate C, and coal C), total N, MBC, and PMN were analyzed by one-way analysis of variance by mine soil type and hydroseed application and combinations. Treatment means when found significantly different by analysis of variance were separated by Tukey's Honest Significant Difference test at the $p \leq 0.05$ level. The R language and Environment for Statistical Computing was used for all analyses (R Development Core Team, 2011).

**4 Results and Discussion**

Detailed annual results of tree survival and growth on the site have been previously reported (Wilson-Kokes et al., 2013b). In brief, these data showed that after six growing seasons (2007-2012), tree survival was significantly higher in brown mine soil treatments compared to gray mine soils (83% vs 72%, data not shown). Average tree volume index (height x diameter$^2$) also was significantly higher for trees grown in brown mine soils compared to gray mine soils after six years and hydroseed treatment doubled the tree volume index on both mine soils (Table 34,780 $cm^3$ vs 1,241 $cm^3$).

Traditionally, aggressive herbaceous vegetation is thought to out-compete tree seedlings for nutrients, water, and solar energy when seeded at high densities (Fields-Johnson et al., 2012). However, when tree-compatible species such as birdsfoot trefoil (*Lotus corniculatus* L.) and ladino clover (*Trifolium repens* L.) are seeded, they can benefit tree seedling growth by reducing evapotranspiration, shading the soil surface, and increasing the soil water-holding capacity through adding organic matter to the soil via root decay (Franklin et al., 2012). In our study, tree survival in hydroseed areas was 82%, which was not significantly different from survival in non-hydroseed areas at 76% (Thomas, 2012). Hydroseed areas showed almost double the tree volume index than non-hydroseed areas (3,953 $cm^3$ vs 2,067 $cm^3$). Hydroseeding by mine soil type resulted in 78% survival and 6,243 $cm^3$ tree volume index on brown and 100% survival and 1,663 $cm^3$ tree volume index on gray (Table 3).

Treatments with hydroseeding initially had significantly higher herbaceous cover with 30% for brown and 22% for gray than those treatments without hydroseeding (3 to 12%, Table 4). Gray mine soils alone had the lowest average total cover at 11% while all other treatment combinations averaged 27 to 39% total cover (Table 4). Perennial ryegrass (*Lolium perenne* L.) and orchardgrass (*Dactylis glomeratus* L.) were the predominant herbaceous species found and accounted for more than 75% of the total herbaceous cover. Legumes in the seeding mix did not establish well and only made up a small part of the herbaceous cover. Hydroseeding was found to be an important component of reclamation because it significantly increased the amount of total cover on hydroseed areas and, as already noted, doubled the tree volume index of trees growing on hydroseed areas compared to non-hydroseed areas.

Birch River mine soils exhibited similar differences in physical and chemical properties as those observed in other studies of brown and gray sandstone mine soils (Angel et al., 2008; Emerson et al., 2009). The pH values for brown mine soils had the lowest mean values of all treatment combinations, 5.0 for brown and 5.5 for brown-hydroseed (Table 5). Gray mine soils ranged in pH from 7.0 to 7.9. The mean pH ranges for brown mine soils in this study fell within the typical range for mine soils created from weathered sandstones (pH 4.5 to 5.5) while the gray mine soils were slightly below to within the typical range for unweathered sandstones (pH 7.5 to 8.0) (Haering et al., 2004; Wilson-Kokes et al., 2013a; Zipper et al., 2013). Compared to initial values measured in 2008, EC values measured in 2012 had not declined substantially, with average ECs ranging from 0.06 to 0.12 dS $m^{-1}$. All EC values were <0.5 dS $m^{-1}$, well below the level at which sensitive species such as sugar maple and white pine experience reduced tree growth and survival. Values of greater than 2 dS $m^{-1}$ have been shown to have detrimental effects on plant growth (McFee et al., 1981; Whiting et al., 2010). Hydroseeding had little effect on soil pH and EC (Table 5).

Percent fines were significantly greater for brown (47 to 58%) vs gray (25 to 42%) mine soils, another common result in similar studies. Miller et al. (2010) found that gray sandstone rocks exhibited a higher durability in a slake-durability test and were highly resistant to weathering during the freeze-thaw test. Zipper et al. (2011) reported that mine soils derived from gray sandstone would continue to have higher coarse fragments and lower percent fines than brown sandstone mine soils as they age because of resistance to degradation. High percentages of coarse fragments reduce water-holding capacities which can negatively influence a site's productivity and tree growth (Rodrigue and Burger, 2004).

Brown mine soils had significantly greater total C and soil organic C than gray mine soils, while carbonate C and coal C were similar among mine soil types (Table 6). Total N was not significantly different among treatments and ranged from 68 to 104 mg $kg^{-1}$~~.~~ (Table 6) and coal N was about 5% of the total N (data not shown). Waschkies and Hüttl (1999) found heterotrophic microflora and fauna were capable of utilizing coal C in mine soil materials, but the levels of N in coal in this study were not sufficient for utilization by plants or microbes (Thomas, 2012).

~~Microbial biomass carbon (MBC) was nearly identical between brown and gray mine soils (13.1 vs 13.0 mg $kg^{-1}$, Table 7). A similar result was found for PMN between brown and gray (0.58 vs 0.57 mg $kg^{-1}$) mine soils.~~ Hydroseed application~~, however,~~ had a significant effect on MBC in brown mine soils and PMN in both mine soils (Table 7). MBC ~~was significantly greater~~ in hydroseed plots~~treatments~~ on brown mine soils had~~with~~ a mass of 17.5~~5.2~~ mg $kg^{-1}$ compared to 8.7~~11.0~~ mg $kg^{-1}$ in non-hydroseed plots. Mean PMN ~~was more than triple~~ in hydroseed treatments was almost triple (0.81~~7~~ mg $kg^{-1}$) that in ~~compared to~~ non-hydroseed (0.35~~29~~ mg $kg^{-1}$). MBC on our gray mine soils were similar varying between 12.8 and 13.3 mg $kg^{-1}$. However, PMN on gray hydroseed plots was more than three times greater at 0.92 mg $kg^{-1}$ compared to non-hydroseed plots having 0.22 mg $kg^{-1}$. In a chronosequence mine soil study, Stephens et al. (2001) found MBC to vary between 266 to 3,351 mg $kg^{-1}$, which was one to three orders of magnitude greater than

MBC in our study. The Stephens et al. study had mine soils varying from 2 to 30 years old, and they were comprised of replaced native soils rather than crushed sandstone materials. A similar two orders of magnitude difference were found for PMN between their study and our study. On the other hand, Littlefield et al. (2013) found small amounts of MBC in reforested mine soils from 1 to 8 years old, which varied from 9 to 57 mg $kg^{-1}$. Their values were much closer to our values, which is probably due to their mine soils being similar in age and constructed from brown and gray sandstone materials like our mine soils. Similar to our findings, Showalter et al. (2010) found that despite differences in soil chemical and physical properties MBC and PMN in brown and gray mine soil materials were not significantly different. According to Schoenholtz et al. (1992), their unseeded sandstone mine soils were lacking in available N for MBC to be very high.

Rice et al. (1996) suggested that the ratio of MBC to total C (MBC:~~/~~TC), as well as PMN to total N (PMN:~~/~~TN), may provide an index of soil organic matter dynamics and soil quality. Some have suggested that mine soils have a large capacity for C storage (Shukla and Lal, 2005; Ussiri et al., 2006, Wick et al., 2009a, 2009b) and these ratios should gradually decline with maturation and time to a stable ratio. In agricultural soils, MBC normally constitutes from 1 to 4% of the total C, while PMN comprises about 2 to 6% of total N (Anderson and Domsch, 1989; Jenkinson, 1988; Sparling, 1992). In mine soils, caution should be exercised in using MBC:~~/~~TC because C may exist as carbonate C and coal C. Over time, the carbonates and coal will weather and degrade, but initially high levels may be found in fresh mine soils. Therefore, in our study we used two ratios: MBC:~~/~~TC and MBC to soil organic C (MBC:~~/~~OC). The MBC:~~/~~OC ratio should be more accurate to correct the error associated with overestimating C contents with C fractions that are not utilized by microorganisms.

The ratio of MBC:~~/~~TC in~~showed~~ brown mine soils was~~to be~~ significantly lower than the other three treatments (0.46 vs an average of 0.93% for the others, Table 7). This result is seemingly contradictory because the brown mine soils with more total C (Table 6) should presumably have ~~had~~ more MBC. However, coal C made up almost half of the total C in these mine soils (Table 6), thereby making almost half of the total C largely unavailable to microbial immobilization. To account for this factor, the ratio of MBC:~~/~~OC should provide a better ratio to assess the percentage of utilizable C used for microbial biomass. ~~When u~~Using this ratio, the brown treatment was still lowest at 1.08~~1~~%~~,~~ with brown hydroseed, ~~and~~ gray was next (1.82 and 2.02%, respectively), and the gray hydroseed was significantly higher at 2.52%). These values are on the low side but within those reported for agricultural soils (1 to 4%, Anderson and Domsch, 1989).

The PMN:TN ratio of non-hydroseed~~ed~~ mine soils in our study were 70 to 80% less than the PMN:TN ratio found in the hydroseed treatments (Table 7), indicating that much less of the N found in the soil was in the active fraction and was therefore not as available to plants and microbes (Stephens et al., 2001). Even though fertilizing with N-$P_2O_5$-$K_2O$ by hydroseeding did not increase the total N (Table 6), fertilizing did increase the PMN and therefore significantly increased~~ing~~ the PMN:~~/~~TN ratio by three or four times. This higher ratio could have been one factor responsible for the much greater tree volume index recorded in hydroseed plots. A regression analysis by Showalter et al. (2010) showed that PMN was highly correlated to tree biomass and therefore the input of litter and nutrients was most likely the limiting factor of growth in mine soils. Perhaps limited N mineralization was responsible for low MBC in non-hydroseed~~ed~~ mine soils of our study, which is further supported by the slightly higher MBC:~~/~~TC ratio found in the hydroseed treatment. This higher ratio indicates that more carbon in the hydroseed treatment was in the active fraction (Insam and Domsch, 1988; Rice et al., 1996).

Hydroseed treatment had a significant effect on biochemical properties of the mine soils due to the earlier fertilization and seeding, which stimulated vegetation growth (Table 4). The resulting greater vegetation and litter provided available C and N for microflora and fauna which contributed to the higher MBC found in the hydroseed treatments. A higher PMN:TN indicated that more of the TN in hydroseed treatments was available to microbes. With this being the only difference between the treatments, we assume that herbaceous vegetation cover was an influential factor in MBC and PMN in these mine soils. Nitrogen-fixing legumes were introduced by hydroseed application, which could have influenced the PMN:TN ratio. The effect of legumes on N cycling is unclear since the total N content of the mine soils with and without hydroseed was not different and the PMN on hydroseed plots was significantly greater in both mine soils. The herbaceous cover contributed by seeded legumes was very low compared to the seeded grasses, therefore it was assumed that the N differences were likely due to the earlier N fertilization.

Mummey et al. (2002) investigated biochemical properties and spatial relationships to plant communities and found that greater MBC, soil organic matter and N depletions were concentrated at the base of plant stems. A similar trend could be occurring at the Birch River site where non-hydroseed plots had little initial soil organic C and N to produce plant growth. Our sampling methods did not take this into account, as soil samples were taken randomly at varying distances from tree bases. We may have diluted our samples by mixing samples near trees with samples further away from trees thereby resulting in lower MBC and PMN. Had we sampled closer to tree bases, which were more abundant on hydroseed areas, we may have found our MBC and PMN values on non-hydroseed plots to be more similar to our hydroseed treatments.

Vegetation that rapidly decomposes and is recycled in mine soils appears to be beneficial for microbial activity. Herbaceous cover may promote a more homogenous soil environment which could promote root expansion by trees and shrubs (Mummey et al., 2002). Other studies on reclamation and biochemical properties have demonstrated the positive impact of mulching and other organic matter additions on biological soil properties of reclaimed mine sites (Anderson et al., 2008; Machulla et al., 2005; Pallavicini et al., 2014; Showalter et al., 2010).

Our study was conducted sixfive years after trees were planted and hydroseeding was performed, and as such provided a snapshot evaluation of reclamation progress at the site. Future studies at this site documenting changes of biochemical properties over time would better assess the nutrient cycling capabilities and restoration of soil quality at the site. In this manner, the success of reclamation practices and the return of ecosystem services could be evaluated. Other studies have examined these dynamic biochemical properties over time and demonstrated the development and evolution of microbial populations and diversity, which were used to indicate reclamation success on a site (Akala and Lal, 2001; Anderson et al., 2008; Chaudhuri et al., 2015; Insam and Domsch, 1988). According to a chronosequence study of reclaimed sites in West Virginia by Stephens et al. (2001), reclaimed mines saw a pulse in microbial activity and biomass in the first 10 years after reclamation, followed by a steady decline during the following years. This pulse was attributed to the rapid consumption of nutrients provided by fertilizers and the quick turnover of herbaceous vegetation and cycling of nutrients. Without additional inputs of nutrients, the plant community was slower to develop, lower amounts of organic materials were added to the soil, thereby resulting in reduced organic matter pools and nutrient cycling because few readily-available nutrients were available for plant and microbial uptake. While PMN and MBC were higher in the hydroseed treatment in this study, it is possible that this site was only experiencing a temporary pulse in microbial activity, which will decline as it approaches 10 years of age.

## 5 Summary and Conclusions

A diverse and active microbial population is essential for sustained primary productivity in ecosystems. Microbes are responsible for the majority of plant litter decomposition and facilitate nutrient cycling through immobilization and mineralization of soil organic compounds. Any drastic land disturbance dramatically alters and disrupts the integrity of the existing plant community, removes the soil resources, and destroys the soil microbial components of an ecosystem. In order to re-establish land capability, reclamation practices must re-establish a soil resource capable of supplying water and nutrients for the plant community and must provide the capacity to support a soil microbial community. With appropriate soil replacement, soil amendments and seeding, the process for developing an ecosystem on the site begins and gradually ecosystem function and stability can occur with time and maturation of the system. Reclamation practices that re-establish the soil, rapidly introduce organic matter, and promote soil microbial populations should be implemented. Brown mine soils were a better growth medium for trees and had higher total and organic C contents compared to gray mine soils. Hydroseeding at a rate of 35 kg $ha^{-1}$ with non-competitive tree compatible herbaceous vegetation and applying 336 kg $ha^{-1}$ of 10-20-10 N-$P_2O_5$-$K_2O$ fertilizer had a significant effect on microbial biomass and activity. On brown mine soils, hydroseeding doubled the amount of MBC, and in both mine soils increased the MBC:/OC and PMN:/TN ratios. Our results indicate that hydroseeding with fertilizer and plant seeds is a useful and beneficial practice to promote plant establishment and to restore soil microbial populations.

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**Table 1.** Species and number of trees planted in 2007 at the Arch - Birch River mine in Webster County, West Virginia.

| Species | Number of trees planted | Percent of trees planted |
|---|---|---|
| Black cherry (*Prunus serotina* Ehrh.) | 880 | 11 |
| Northern red oak (*Quercus rubra* L.) | 880 | 11 |
| Sugar maple (*Acer saccharum* Marsh.) | 880 | 11 |
| White ash (*Fraxinus americana* L.) | 880 | 11 |
| White oak (*Quercus alba* L.) | 880 | 11 |
| Black locust (*Robinia psuedoacacia* L.) | 800 | 10 |
| Pitch X loblolly pine (*Pinus X rigitaeda*) | 800 | 10 |
| Tulip-poplar (*Liriodendron tulipifera* L.) | 640 | 8 |
| Sycamore (*Platanus occidentalis* L.) | 480 | 6 |
| Eastern white pine (*Pinus strobus* L.) | 400 | 5 |
| Dogwood (*Cornus alternifolia* L.) | 320 | 4 |
| Eastern redbud (*Cercis canadensis* L.) | 320 | 4 |
| Total | 8,160 | 100 |

**Table 2.** Species and rate of application of ground cover hydroseeded in 2008 at the Arch - Birch River mine in Webster County, West Virginia.

| Species | Rate |
|---|---|
| | kg $ha^{-1}$ |
| Birdsfoot trefoil (*Lotus corniculatus* L.) | 11.2 |
| Kobe lespedeza (*Kummerowia striata* Maxim.) | 5.6 |
| Ladino clover (*Trifolium repens* L.) | 3.3 |
| Orchard grass (*Dactylis glomerata* L.) | 5.6 |
| Perennial ryegrass (*Lolium perenne* L.) | 5.6 |
| Redtop (*Agrostis gigantea* Roth) | 2.2 |
| Weeping lovegrass (*Eragrostis curvula* Schra.) | 2.2 |
| Total | 35.7 |

**Table 3.** Mean tree volume index[Ɨ] for 2008-2012 growing seasons in four soil treatments at the Arch - Birch River mine in Webster County, WV (Wilson-Kokes et al., 2013b).

| | Treatments | | | |
|---|---|---|---|---|
| Year | Brown | Brown-Hydroseed | Gray | Gray-Hydroseed |
| | ---------- | ---------- $cm^3$ | ---------- | ---------- |
| 2008 | 43[a €] | 50[a] | 27[a] | 17[a] |
| 2009 | 218[a] | 293[a] | 30[b] | 40[b] |
| 2010 | 582[ab] | 712[a] | 72[c] | 229[bc] |
| 2011 | 2086[a] | 1687[a] | 248[b] | 163[b] |
| 2012 | 3316[ab] | 6243[a] | 818[c] | 1663[bc] |

[Ɨ] Tree volume index determined by tree height and diameter$^2$ (measured at 5 cm above ground).
[€] Means for each volume within rows (years) with the same letter are not significantly different at $p < 0.05$.

**Table 4.** Mean ground cover on four soil treatments in 2012 at Arch - Birch River mine in Webster County, WV (Wilson-Kokes et al., 2013b).

| | Treatments | | | |
|---|---|---|---|---|
| Cover | Brown | Brown-Hydroseed | Gray | Gray-Hydroseed |
| | ---------- | ---------- % | ---------- | |
| Herbaceous | 12[c Ɨ] | 30[a] | 3[d] | 22[b] |
| Tree | 15[a] | 9[a] | 8[a] | 9[a] |
| Total Cover | 27[b] | 39[a] | 11[c] | 29[b] |
| Bare/Rock | 73[b] | 61[c] | 89[a] | 69[b] |

[Ɨ] Means for each treatment combination within rows with the same letter are not significantly different at $P < 0.05$.

**Table 5.** 2008 and 2012 soil properties of samples from four soil treatments at the Arch - Birch River mine in Webster County, WV (Wilson-Kokes et al., 2013b).

| | Treatments | | | |
|---|---|---|---|---|
| Property | Brown | Brown-Hydroseed | Gray | Gray-Hydroseed |
| pH | | -------------- su ------------ | | |
| 2008 | 4.7[b Ɨ] | 4.8[b] | 7.9[a] | 7.4[a] |
| 2012 | 5.0[b] | 5.5[b] | 7.0[a] | 7.7[a] |
| EC | | -------------- dS m$^{-1}$ ---------- | | |
| 2008 | 0.12[a] | 0.06[b] | 0.12[a] | 0.12[a] |
| 2012 | 0.06[b] | 0.07[b] | 0.11[a] | 0.09[ab] |
| Fines[€] | | ----------------- % -------------- | | |
| 2008 | 58[a] | 47[b] | 42[b] | 34[c] |
| 2012 | 63[a] | 55[ab] | 40[b] | 25[c] |

[Ɨ] Means for each treatment combination within rows with the same letter are not significantly different at P < 0.05.

[€]Material that passed a 2 mm sieve.

**Table 6.** Carbon and n~~N~~itrogen fractions in mine soil samples after six~~five~~ growing seasons at the Birch River Operation in Webster County, WV.

| | Treatments | | | |
|---|---|---|---|---|
| Property | Brown | Brown-Hydroseed | Gray | Gray-Hydroseed |
| | | ---------------------- mg kg$^{-1}$ -------------------- | | |
| Total C | 1880[a Ɨ] | 1760[a] | 1412[b] | 1463[b] |
| Soil organic C | 806[a] | 964[a] | 659[b] | 507[b] |
| Carbonate C | 346[a] | 185[a] | 240[a] | 323[a] |
| Coal C | 728[a] | 611[ab] | 513[b] | 633[ab] |
| Total N | 104[a] | 75[a] | 96[a] | 68[a] |

[Ɨ] Means for each treatment combination within rows with the same letter are not significantly different at P < 0.05.

**Table 7.** Biochemical properties and ratios for mine soils after six~~five~~ growing seasons at the Birch River Operation in Webster County, WV.

| | Treatments | | | |
|---|---|---|---|---|
| Property | Brown | Brown-Hydroseed | Gray | Gray-Hydroseed |
| | ----------------------- mg kg$^{-1}$ ---------------------- | | | |
| MBC[Ɨ] | 8.7[c,€] | 17.5[a] | 13.3[b] | 12.8[b] |
| PMN | 0.35[b] | 0.81[a] | 0.22[b] | 0.92[a] |
| | ----------------------- % ---------------------- | | | |
| MBC:~~/~~TC | 0.46[b] | 0.99[a] | 0.94[a] | 0.87[a] |
| MBC:~~/~~OC | 1.08[c] | 1.82[b] | 2.02[b] | 2.52[a] |
| PMN:~~/~~TN | 0.34[b] | 1.08[a] | 0.23[b] | 1.35[a] |

[Ɨ] MBC - Microbial biomass carbon, PMN - Potentially mineralizable nitrogen.
[€] Means for each treatment combination within rows with the same letter are not significantly different at $P < 0.05$.