MICROBIAL INDICATORS OF MINESOIL QUALITY IN SOUTHERN WEST VIRGINIA

by

K.M. Stephens, A.J. Sexstone, J.C. Sencindiver, J.G. Skousen, and K.A. Thomas

Abstract. As coal mining by mountaintop removal has become more prominent in southern West Virginia, questions have arisen about its effect on soil and water quality. Reclaimed mined lands normally have drastically different soil properties as compared to contiguous native soils, so there is concern about development and productivity of these soils. The destruction of the A horizon where mining occurs results in reclaimed minesoils having low organic matter content and low microbial activity. A study was conducted to evaluate changes in microbial properties of reclaimed minesoils over time. Minesoils on three different sites, Hobet-21, Cannelton, and Dal-Tex, were sampled. Two ages were sampled at the Hobet (8 and 17 years) and Cannelton (16 and 30 years) sites. Four ages (2, 7, 11, and 23 years) were sampled at the Dal-Tex site. Contiguous native soils also were sampled at each site. Soil pits were dug to a depth of 40 cm, and the bulk samples were removed from the A horizons. Texture, pH, bulk density, electrical conductivity, and total C, N, and S were determined for each sample. Also, microbial biomass C (MBC) and N (MBN), potentially mineralizable N (PMN), and microbial respiration (MR) were determined. Microbial properties of minesoils and native soils were generally within the ranges reported for other forest, agricultural, and reclaimed soils. Ratios of MBC to total C (TC), MBN to total N (TN), PMN to TN and MR to MBC indicate that microbial activity stabilizes and organic matter accumulation and stabilization increases with minesoil age. Therefore, soil quality appears to be improving with time in mine soils from mountaintop removal mining areas.

Additional Key Words: Reclamation, Mountaintop mining, Mud River, Twentymile Creek, Spruce Fork

Introduction

Soil quality has been defined as the capacity of the soil, within land use and ecosystem boundaries, to sustain biological productivity, maintain environmental quality, and promote plant health (Doran et al., 1999). Doran and Parkin (1996) proposed a data set of soil physical, chemical, and microbial properties that provide quantitative indicators of a soil's quality (1996). Periodic measurement of these properties at a specific site provides evidence that soil quality is either improving or degrading over time.

Minesoil quality is important not only for initial revegetation but also for continued long-term productivity and environmental quality. Mountaintop removal coal mining drastically changes soil quality. Following reclamation however, the resultant minesoils are subject to similar factors and processes that developed the surrounding contiguous undisturbed soils. With sufficient time, minesoils have the potential to improve to a level of soil quality similar to that present prior to mining.

Microorganisms are sensitive biological markers that may be useful in documenting early stages of recovery in disturbed systems such as minesoils (Turco et al., 1994). Therefore, the objective of this study was to document differences in microbial properties of minesoils on reclaimed mountaintop removal coal mines, with particular emphasis on measurement of microbial indicators of soil quality including microbial biomass carbon, microbial biomass nitrogen, potentially mineralizable nitrogen, and microbial respiration.

---

[1]Paper presented at the 2001 National Meeting of the American Society for Surface Mining and reclamation, Albuquerque, New Mexico, June 3-7, 2001. Pub. by ASSMR, 3134 Montavesta Rd., Lexington, KY 40502.

[2]K.M. Stephens and K.A. Thomas are graduate research assistants, A.J. Sexstone is Associate Professor and J.C. Sencindiver and J.C. Skousen are Professors in the Division of Plant and Soil Sciences, West Virginia University, P.O. Box 6108, Morgantown, WV 26506-6108.

Proceedings America Society of Mining and Reclamation, 2001 pp 317-325
DOI: 10.21000/JASMR01010317

https://doi.org/10.21000/JASMR01010317

Methods and Materials

Site Descriptions and Sampling Procedure

Minesoil samples were collected from three reclaimed mountaintop removal coal mines in southern West Virginia. The Hobet-21 mine is located in the Mud River watershed in Boone County, the Cannelton mine is located in the Twentymile Creek watershed of Fayette County, and the Dal-Tex mine is located in Spruce Fork watershed in Logan County. Two different ages of minesoils on gentle slopes were selected for sampling at the Hobet-21 (8 and 17 year-old-soils) and Cannelton sites (16 and 30 year-old-soils). These minesoils developed under similar geology, topography, and climatic conditions, and were subjected to similar mining and reclamation methods. Three sampling points (replications) were located on each of the minesoil sites. In addition, three sampling points were located on the contiguous strongly sloping native soils. The soil series mapped at these sites were: Muskingum (fine-loamy, mixed, mesic Typic Dystrochrepts) at Cannelton and Berks (loamy-skeletal, mixed, mesic Typic Dystrochrepts) and Gilpin (fine-loamy, mixed, mesic Typic Hapludults) at Hobet-21 (Gorman and Espy, 1975; Wolf, 1994). Sampling points were spaced at intervals of 250 m at each site.

Four different ages of minesoils (23, 11, 7, and 2 years old) were sampled on the Dal-Tex site. Three gently sloping and three strongly sloping sampling points (replications) were located on each site. Two sampling points were located on strongly sloping native soils. The soils at these two sites were classified as the Berks series (loamy-skeletal, mixed, mesic Typic Dystrochrepts) and the Matewan series (loamy-skeletal, mixed, mesic Typic Dystrochrepts). Distance between sampling points on this mine differed for each age of soil. Each of the sampling points at the 2-year-old site was within a distance of 20 m from the next point. Sampling points on the native soils and on each of the other minesoil ages were more then 20 m apart. The longest distance between points was approximately 100 meters on the 23-year-old site.

All native soils at each of the sites were forested. Both minesoil sites at Cannelton were predominantly vegetated with grasses and legumes with some scattered trees. The 16-year-old site had scattered black locust (*Robinia pseudoacacia*), but the 30-year-old site had more trees of a variety of species including black locust, maples (*Acer* sp.), pines (*Pinus* sp.), sweet gum (*Liquidambar stryaciflua*) and sourwood (*Oxydendrum arboreum*). The 8-year-old site at Hobet-21 was covered with grasses and legumes. The major cover on the Hobet-21, 17-year-old site was black locust with a ground cover of grasses and legumes. At Dal-Tex, the 23-year-old site was primarily forested with some grasses and legumes on the gently sloping sites. The 7-year-old site predominantly had grasses and legumes with some shrubs. The 2-year-old site was covered with grasses and legumes. The major vegetation at the 11-year-old site was grasses and legumes with the addition of a few scattered trees.

In early June 2000, which is late spring in West Virginia, a soil pit was dug to a depth of 40 cm or more at each sampling point, allowing for full exposure of the upper mineral horizon and one or more subsurface horizons. The soil was described to the exposed depth, and bulk samples were collected from the surface horizon for laboratory analyses. All bulk samples were immediately stored at 4° C until analyzed. Bulk density of the surface horizon was determined in the field by a frame excavation technique developed by soil scientists at the Natural Resources Conservation Service National Soil Survey Laboratory in Lincoln, NE (R.B.Grossman, unpublished procedure).

Laboratory Analyses

All samples were processed through a 2 mm sieve before analysis. Texture, pH, and electrical conductivity (EC) were determined by standard methods of the National Soil Survey Laboratory (Soil Survey Staff, 1996). A LECO CNS-2000 analyzer was used to determine total carbon, nitrogen, and sulfur. It was assumed that total soil C was equivalent to total organic soil C. Microbial biomass C and N were determined by the CFE procedure (Rice et al., 1996). Twenty grams of soil, weighed at field moisture content, were used for this procedure. Total N in extracts was determined by flow injection analysis (Lachat QuickChem 8000 Series), and organic C was measured by a Tekmar-Dohrman DC-190 automated carbon analyzer. Potentially mineralizable N was determined by the anaerobic incubation procedure (Drinkwater et al., 1996). Eight grams of soil were weighed before placement in a centrifuge tube for analysis. Ammonium and nitrate-nitrogen were determined in extracts by flow injection analysis (Lachat QuickChem 8000 Series). For both the microbial biomass and N mineralization potential, preweighed soil samples were removed from 4° C storage and placed in dark conditions at room temperature for 24 hr to allow for any pulse of heterotrophic microbial activity.

Microbial respiration was determined by static soil incubation in closed bottles (Zibilske et al., 1994). Triplicate soil samples (25 g field moist) were placed in funnels lined with Whatman #1 filter paper. Soils were then completely saturated with 100 ml of distilled water and allowed to drain for 24 hr to normalize soil moisture. Wetted soil (20 g) was weighed into serum bottles (160 ml) and incubated uncovered at room temperature in the dark for 24 hr. Each bottle was capped with a butyl rubber stopper, and initial headspace CO_2 levels were established by injecting 1 ml via a syringe into an infrared gas analyzer (IRGA) equipped with a gas recirculation loop. This process was repeated for each bottle at 24, 48, 72, and 96 hr. Microbial respiration rates were determined using linear regression analysis of CO_2 concentrations at each sampling time.

Results and Discussion

Physical and chemical properties of the minesoils and native soils are presented in Table 1. Most soils had loamy textures, i.e. sandy loam, loam, silt loam, or silty clay loam with clay contents ranging from 10.0-21.6%. Electrical conductivity values were low for all soils, reflecting the low pyrite content of the unconsolidated material and indicating that formation of acid salts upon weathering should not limit site revegetation. Minesoil pH ranged from 4.4 on the 23-year-old strongly sloping Dal-Tex site to 6.3 on the 8-year-old Hobet-21 site. Native soil pH values ranged from 4.2 to 5.0. Low total S values for all minesoils and native soils in this study were similar to values reported by Smith et al. (1976) for soils and overburden in nearby Mingo County, WV.

Minesoil total C and total N values were similar to other minesoils having comparable vegetation (Li, 1991). An exception was the minesoil at the Cannelton site with 10% organic carbon. At the Dal-Tex site, total C and N values tended to be lower in minesoils regardless of age compared to native soils. Younger minesoils (2 and 7 years) at the Dal-Tex site had higher total C but not total N, compared to older minesoils (11 and 23 years). However, minesoils on the Cannelton and Hobet sites always had higher total C and total N values compared with native soils. Although in physical proximity, native soils usually were situated on steeper slopes, retained their well-developed cover of native trees, and were generally less compacted than the reclaimed minesoils. These data highlight the difficulty in strict comparisons between minesoil properties and those of adjacent native soils.

Heterotrophic microbial growth and activity in a soil often correspond to available C and inorganic soil nutrients, particularly N. Microbial biomass is a labile fraction of the soil organic matter and it exhibits a rapid turnover time compared to more stable humic materials. It also functions as a source and sink of available soil nutrients (Srivastava, 1992). Schafer et al. (1979) suggested that microbial properties of a reclaimed minesoil would reach the levels found in native soils at a faster rate than total organic matter content. To test this, soil MBC, MBN, PMN, and MR for minesoils and native soils were measured (Table 2). Reported values generally were within published ranges for forest and agricultural soils and reclaimed minesoils (Myrold, 1987; Rice et al., 1996; Insam and Domsch, 1988). Minesoil MBC values generally were higher than values reported by Bonde et al. (1988) from long-term cropping experiments; however MBN and PMN values were similar. The native soils at Dal-Tex and Cannelton generally exhibited similar microbial parameters, but values for the Hobet native soil were lower. The reasons for this difference are not immediately apparent because soils and vegetation were similar at each of the three native sites.

Cannelton sites exhibited the strongest differences between individual microbial measurements and minesoil age. The 30-year-old Cannelton soil exhibited the highest potential microbial activity of all soils in this study. The silt loam, low bulk density soil at the 30-year old Cannelton site was higher in total carbon and nitrogen than the other minesoils (Table 1). This soil was located in a lower-lying landscape position than the other minesoils, so its profile remained wetter during the growing season, thereby favoring accumulation of organic matter and supporting an active microbial community. Dal-Tex and Hobet minesoils showed less correlation between individual microbial measurements and minesoil age than did Cannelton. However, values reported for the 2-year-old Dal-Tex minesoils were significantly different based upon one standard error when compared both to older minesoils and adjacent native soils (Table 2). The magnitude of the differences depended on the position of the sites. Two-year-old Dal-Tex soils on gently sloping sites had significantly lower values for microbial measurements compared to other soils. Two-year-old Dal-Tex soils on strongly sloping sites generally had higher values for microbial measurements than any other minesoil. The latter case probably is explained by relatively recent additions of organic matter and fertilizers following reclamation. Soils on the gentle slope had higher corrected bulk densities and lower C, N, and S concentrations than their strongly sloping counterparts.

Table 1. Minesoil physical and chemical properties of the A horizons.

Soil ID	pH	EC	Bulk Density		Texture				Total		
			Uncorrected	Corrected	Sand	Silt	Clay	Class[1]	Carbon	Nitrogen	Sulfur
		dS/m	g/cm3	g/cm3	------------%----------				---------------%---------------		
Dal-Tex											
Gently sloping											
23-year-old	4.8	0.13	1.10	0.86	49.5	35.1	15.4	L	3.96	0.16	0.06
11-year-old	5.5	0.18	1.59	1.18	61.3	26.5	12.2	SL	2.61	0.04	0.05
7-year-old	5.4	0.13	1.47	0.98	42.4	36.8	20.8	L	4.58	0.04	0.09
2-year-old	5.4	0.11	1.37	1.05	52.9	32.1	15.0	SL	3.10	0.00	0.04
Strongly sloping											
23-year-old	4.4	0.09	1.28	0.88	53.2	30.6	16.2	SL	2.21	0.04	0.03
11-year-old	5.1	0.08	1.44	1.02	57.8	29.8	12.4	SL	1.60	0.01	0.03
7-year-old	4.8	0.09	1.39	0.94	57.6	28.6	13.7	SL	3.70	0.01	0.06
2-year-old	6.1	0.14	1.54	0.92	51.6	32.9	15.5	L	5.02	0.05	0.09
Undisturbed	4.2	0.29	0.72	0.61	40.8	44.7	14.6	L	9.04	0.28	0.07
Cannelton											
Gently sloping											
30-year-old	4.9	0.20	0.58	0.39	15.8	62.7	21.6	SIL	10.12	0.54	0.09
16-year-old	6.3	0.08	1.78	1.23	37.0	42.8	20.2	L	2.29	0.02	0.05
Strongly sloping											
Undisturbed	5.0	0.09	0.92	0.64	32.7	50.1	17.2	SiL	3.99	0.16	0.03
Hobet-21											
Gently sloping											
17-year-old	6.0	0.30	1.02	0.69	28.4	53.1	18.5	SiL	9.52	0.45	0.13
8-year-old	6.2	0.11	1.10	0.85	42.8	43.6	13.7	L	5.40	0.14	0.08
Strongly sloping											
Undisturbed	4.7	0.07	0.85	0.58	53.4	36.6	10.0	SL	3.32	0.06	0.02

[1]-SiL=Silt Loam; SiCL=Silty Clay Loam; SL=Sandy Loam; LS=Loam Sand; L=Loam

Table 2. Minesoil microbial biomass carbon and nitrogen, potentially mineralizable nitrogen, and Microbial respiration of the A horizons.

Soil ID	Microbial Biomass Carbon	Microbial Biomass Nitrogen	Potentially Mineralizable Nitrogen	Microbial Respiration
	mg/kg[1]	mg/kg[1]	mg/kg[1]	µg CO_2-C/kg/hr[1]
Dal-Tex				
Gently Sloping				
23-year-old	950 ±146	77±13	94±13	1132±195
11-year-old	945±82	71±23	144±25	1638±278
7-year-old	1142±184	96±26	200±24	2772±604
2-year-old	266±48	17±2	38±4	166±48
Strongly Sloping				
23-year-old	524±70	26±5	68±13	798±314
11-year-old	616±68	42±7	90±8	1199±262
7-year-old	489±202	47±16	59±38	944±530
2-year-old	1022±126	70±13	170±36	1312±743
Undisturbed	1528±357[2]	114±24[2]	68±14[2]	1414±426[2]
Cannelton				
Gently Sloping				
30-year-old	3351±793	329±91	308±46	4137±1010
16-year-old	280±30	26±7	37±6	247±27
Strongly Sloping				
Undisturbed	1029±74	119±16	68±6	796±142
Hobet-21				
Gently Sloping				
17-year-old	1928±524	190±60	152±35	1819±569
8-year-old	1166±235	108±28	111±4	833±96
Strongly Sloping				
Undisturbed	883±65	69±3	53±4	592±57

[1]-Values expressed as the mean plus or minus the standard error, with n=3.
[2]–Mean and standard error based on n=2.

A third order ploynomial regression was conducted for each microbial property verses age. With 0.10 signifigance level we reconstituted the means of MBC, MBN, and, MR, using the third order polynomial (Figure 1). The reconstituted means of PMN are not presented in Figure 1 due to lack of sigifigance between age and this property. Reconstituted means of MBC, MBN and MR showed a steady increase over the first ten years after reclamation, followed by a decrease over the next ten years. The values estimated over the last few years sharply increased, indicating a movement towards native soil conditions. The intial pulse of microbial activity may be explained by the slow depletion of organic matter and fertilizers added at the time of reclaimation. As these materials were being incorporated into the soil, microbial activity was elevated. The decline in values over the following ten years indicate that added nutrients and organic matter have been depleted from the A horizons and the soil was becoming dependent on natural cycles. During this period, the soils were somewhat depleted of necessary nutrients leading to low microbial activity. Yet, as the soil continued to incorporate organic materials into the A horizons, the nutirent pools were stenghtened, as indicated by the rise in microbial activity over the last three years.

Rice et al. (1996) suggested that the ratio of microbial biomass C and N to total soil organic C and N (MBC/TC and MBN/TN) may provide measures of soil organic matter dynamics and soil quality. Mature soils often reach a steady state where ratios of these values are reasonably constant. In agricultural soils, MBC normally comprises 1 to 4% of the total organic C, and MBN comprises 2 to 6% of the total organic N (Anderson and Domsch, 1989; Jenkinson, 1988; Sparling, 1992).

Ratios of MBC/TC and MBN/TN obtained from minesoils are potentially complicated by the presence of carbolithic rock fragments which could inflate values of both total C and total N (Headlee, 1955). In the present study, the MBC/TC ratios obtained from minesoils and native soils ranged from 0.9 to 3.9%, similar to values reported above for agricultural soils (Table 3). However MBN/TN ratios had a much greater range from 4.1 to 47% (Table 3). The disparity between the two ratios could be due to higher C values from carbolithic shales and rock fragments present in the minesoils. Reeder (1988) has indicated that minesoils contain large quantities of organic C in inert forms that are resistant to microbial utilization.

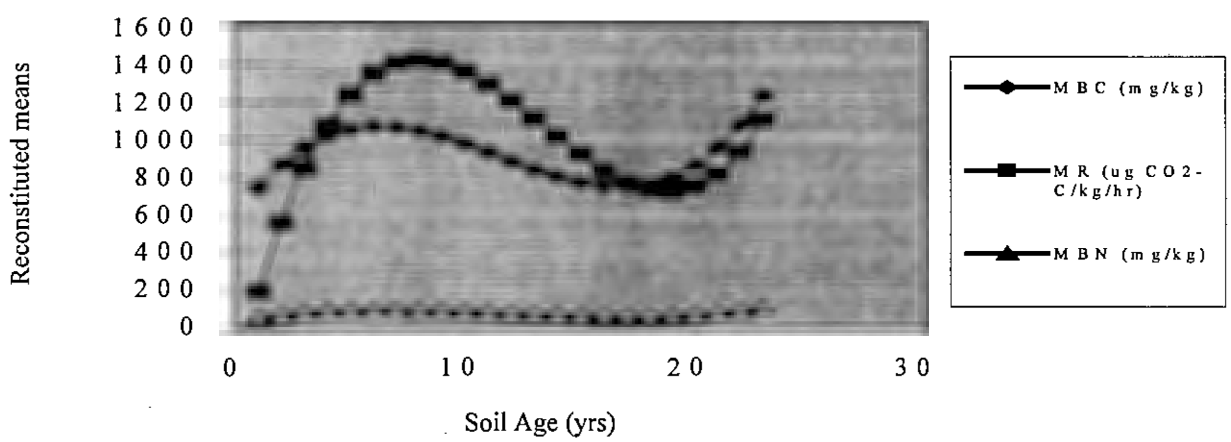

Figure 1. Reconstituted means of microbial biomass carbon (MBC), microbial respiration (MR), microbial biomass nitrogen (MBN) based on a third order regression of age verses MBC, MR, and, MBN.

Table 3. Ratios of microbial biomass C (MBC) to total C (TC), microbial biomass N (MBN) to total N (TN), potentially mineralizable N (PMN) to TN, and microbial respiration (MR) to MBC on native soils and minesoils at the Dal-Tex site, Cannelton site, and the Hobet-21 site.

Soil ID	Slope Class[1]	MBC/TC	MBN/TN	PMN/TN	MR/MBC
		%	%	%	CO_2-C/hr x10^{-4}
Dal-Tex					
Undisturbed	SS	1.7	4.1	2.4	9.2
23-year-old	GS	2.4	4.8	5.8	11.9
	SS	2.4	6.5	16.8	15.2
11-year-old	GS	3.6	17.8	35.9	17.3
	SS	3.9	42.0	90.4	19.5
7-year-old	GS	2.5	24.0	50.0	24.3
	SS	1.3	47.0	84.7	19.3
2-year-old	GS	0.9	--	--	6.2
	SS	2.0	14.0	33.9	12.8
Cannelton					
Undisturbed	SS	2.5	7.4	4.2	7.7
30-year-old	GS	3.3	6.1	5.7	12.3
16-year-old	GS	1.2	13.1	18.3	8.8
Hobet-21					
Undisturbed	SS	2.7	11.4	8.8	6.7
16-year-old	GS	2.0	4.2	3.4	9.4
8-year-old	GS	2.2	7.7	7.9	7.1

[1]-GS=Gently Sloping; SS=Strongly Sloping

Therefore the N values and ratios may provide more reliable site comparisons (Drinkwater et al., 1996; Coyne et al., 1998). Total N values for all sites were low, which may indicate that N-leaching and plant N-uptake were significant fates of nitrogen in these soils.

As the humic content of the soil organic matter pool increases, soil organic carbon and nitrogen become less susceptible to microbial mineralization. Therefore with time, ratios of MBC/TC and MBN/TN and PMN/TN should decrease. This relationship probably does not apply to recently reclaimed sites where rapidly decomposing organic matter and recent fertilization events complicate the ratios. Excluding the two-year-old soil, these chronological trends are apparent at the Dal-Tex site. The MBN/TN and PMN/TN ratios generally decrease in the following order: 7 years > 11 years > 23 years > native soil. For the MBC/TC ratios, there is a decrease in the following order: 11 years > 7 years = 23 years > native soil. These data suggest there is stabilization and accumulation of soil organic matter with age in these soils. The same relationship of decreasing ratios with age generally were evident at the Cannelton but not the Hobet sites. However, examination of additional soils of different ages would be necessary to draw conclusions about chronological trends at these sites.

Soil respiration previously has been used to assess decomposition dynamics in West Virginia minesoils (Stroo and Jencks, 1985). Kennedy and Papendick (1995) suggested that a respiratory quotient such as the MR/MBC ratio relates both the size and activity of microbial biomass. A lowering of the ratio indicates a trend to a more stable and mature system (Insam and Domsch, 1988). The respiratory quotient for the Dal-Tex soils decreased in the following order: 7 years> 11 years> 23 years> native soil. Again excluding the 2-year-old soil, this trend indicated a maturation of soils at the Dal-Tex site. A decreasing respiratory quotient with site age was not observed at the Cannelton and Hobet sites.

Our data measured an accumulation of C and N in stable organic matter with increasing minesoil age. The older minesoils, especially the 23-year-old minesoils at the Dal-Tex and the 30-year-old minesoils at Cannelton, had properties similar to native soils. These data and companion data by Thomas et al. (2000) indicate that soil quality of minesoils examined in this study was increasing with time.

Acknowledgements

We appreciate the assistance of Mr. Rob Pate, soil scientist with the Natural Resources Conservation Service, for his assistance in selecting native soil sites and describing minesoils. This research was supported by funds provided by Arch Coal, Inc. and the West Virginia Legislature, and by funds appropriated under the Hatch Act. West Virginia Agricultural and Forestry Experiment Station Scientific Article No. 2779.

Literature Cited

Anderson, J.P.E., and K.H. Domsch. 1989. Ratios of microbial biomass carbon to total carbon in arable soils. Soil Biol. Biochem. 21: 471-479.
https://doi.org/10.1016/0038-0717(89)90117-X

Bonde, T.A., J. Schnurer, and T. Rosswall. 1988. Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. Soil Biol. Biochem. 20:447-452.
https://doi.org/10.1016/0038-0717(88)90056-9

Coyne, M.S., Q. Zhai, C.T. Mackown, and R.I. Barnhisel. 1998. Gross nitrogen transformation rates in soil at a surface coal mine site reclaimed for prime farmland use. Soil Biol. Biochem. 30: 1099-1106.
https://doi.org/10.1016/S0038-0717(97)00202-2

Doran, J.W., A.J. Jones, M.A. Arshad, and J.E. Gilley. 1999. Determinants of soil quality and health. Chapter 2. p. 17-36. *In* R. Lal (ed.), Soil Quality and Soil Erosion. CRC Press. Boca Raton, Florida.

Doran, J.W. and T.B. Parkin. 1996. Quantitative indicators of soil quality: a minimum data set. Chapter 2. p. 25-37. *In* J.W Doran and A.J. Jones (eds.). Methods for Assessing Soil Quality. SSSA Spec. Publ. No. 49. Soil Science Society of America, Inc. Madison, WI.

Drinkwater, L.E., C.A. Cambardella, J.D. Reeder, and C.W. Rice. 1996. Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen. Chapter 13, p. 217-229. *In* J.W Doran and A.J. Jones (eds.). Methods for Assessing Soil Quality. SSSA Spec. Publ. No. 49. Soil Science Society of America, Inc. Madison, WI.

Gorman, J.L., and L.E. Espy. 1975. Soil Survey of Fayette and Raleigh Counties, West Virginia. USDA Soil Conservation Service. Washington, DC.

Headlee, A.J.W. 1955. Characteristics of mineable coals of West Virginia. West Virginia Geological Survey. Pt. VI. The structure of bituminous coals. 13(A). 123-149.

Insam, H. and K.H. Domsch. 1988. Relationship between soil organic carbon and microbial biomass on chronosequences of reclaimed sites. Microbiol. Ecol. 15:177-188.

Jenkinson, D.S. 1988. Determination of microbial biomass carbon and nitrogen in soil. p. 368-386. *In* J.R. Wilson (ed.) Advances in nitrogen cycling in agricultural ecosystems. CAB Int., Wallingford, England.

Kennedy, A.C. and R.I. Papendick. 1995. Microbial characteristics of soil quality. J. Soil and Water Conservation 50:243-248.

Li, R. 1991. Nitrogen cycling in young mine soils in southern Virginia. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. Blacksburg, VA. 150 p.

Myrold, D.D. 1987. Relationship between microbial nitrogen and a nitrogen availability index. Soil Sci. Soc. Am. J. 51:1047-1049.
https://doi.org/10.2136/sssaj1987.03615995005100040040x

Reeder, J.D. 1988. Transformations of nitrogen-15-labeled fertilizer nitrogen and carbon mineralization in incubated coal mine spoils and disturbed soil. J. Environ. Qual. 17:291-298.
https://doi.org/10.2134/jeq1988.00472425001700020022x

Rice, C.W., T.B. Moorman, and M. Beare. 1996. Role of microbial biomass carbon and nitrogen in soil quality. Chapter 12. p. 203-215. *In* J.W Doran and A.J. Jones (eds.). Methods for Assessing Soil Quality. SSSA Spec. Publ. No. 49. Soil Science Society of America, Inc. Madison, WI.

Schafer, W.M., G.A. Nielson, D.S. Dollhopf, K. Temple. 1979. Soil genesis, hydrological properties, root characteristics, and microbial activity of 1 to 50-year-old strip mine spoils. EPA-600/ 7-79-100. Environmental Protection Agency. Cincinnati, OH.

Smith, R.M., A.A. Sobek, T. Arkle, Jr., J.C. Sencindiver, and J.R. Freeman. 1976. Extensive overburden potentials for soil and water quality. EPA-600/2-76-184. National Technical Information Service. Springfield, VA.

Soil Survey Staff. 1996. Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42. Version 3.0. USDA Natural Resources Conservation Service. Lincoln, NE.

Sparling, C.P. 1992. Ratio of microbial biomass to soil organic carbon as a sensitive indicator of changes in soil organic matter. Aust. J. Soil Res. 30:195-207.
https://doi.org/10.1071/SR9920195

Stroo and Jencks. 1985. Effect of sewage sludge on microbial activity in an old abandoned minesoil. J. Environ. Qual. 14:301-304.
https://doi.org/10.2134/jeq1985.00472425001400030001x

Thomas, K.A., J.C. Sencindiver, J.G. Skousen, and J.M. Gorman. 2000. Soil development on a mountain top removal mine in southern West Virginia. p. 546-556. *In* W.L. Daniels and S.G. Richardson (eds.). Proc. Seventeenth Annual Meeting, American Society for Surface Mining and Reclamation. 11-15 June 2000. Tampa, FL. Amer. Soc. Surf. Mining Rec., 3134 Montavesta Rd., Lexington, KY.
https://doi.org/10.21000/JASMR00010546

Turco, R.F., A.C. Kennedy, and M.D. Jawson. 1994. Microbial indicators of soil quality. Chapter 5. p. 73-90. *In* J.W. Doran, D.C. Coleman, D.F. Bezdicek, and B.A. Stewart (eds.) Defining Soil Quality for a Sustainable Environment. SSSA Spec. Publ. No. 35. Soil Science Society of America. Madison, WI.

Wolf, B.L. 1994. Soil Survey of Boone County, West Virginia. USDA Soil Conservation Service. Washington, DC.

Srivastava, S.C. 1992. Microbial C, N and P in dry tropical soils: seasonal changes and influence of soil moisture. Soil Biol. Biochem. 24:711-714.
https://doi.org/10.1016/0038-0717(92)90050-8

Zibilske, L.M. 1994. Carbon mineralization. Chapter 38. p. 835-863. *In* Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. SSSA Book Series No. 5. Soil Science Society of America. Madison, WI.