SHORT-TERM MICROBIAL RESPIRATION AS AN INDICATOR OF SOIL QUALITY FOR RECLAIMED COAL MINE SOILS OF NORTHEASTERN WYOMING[1]

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Abstract. Soil quality and the ability of soil to sustain nutrient cycling in reclaimed soils will influence the subsequent establishment and maintenance of a permanent and stable plant community. We undertook an experiment using a recently developed "three-day CO_2 flush method" to compare a range of soil biological indicators across a series of reclaimed, surface coal-mined sites in the Powder River Basin of northeastern Wyoming. In addition, we were interested in estimating the amount of soil organic carbon (SOC) required to sustain nutrient cycling. Soils were sampled from each of two different reclaimed sites on four different mines in 2000. In 2001 we sampled soils from three sites on three mines - two reclaimed and a native, undisturbed prairie control site. For both years, soils were dried, rewetted, and microbial respiration measured at three and 21 days, using base trap methods. In addition, microbial biomass, nitrogen (N)-mineralization, organic carbon (C) and total N were measured. Regression analyses were accomplished by regressing three-day microbial respiration against the other soil parameters measured. Correlations between three-day microbial respiration and all of the measured soil parameters were generally strong ($r^2 \geq 0.55$) and highly significant ($P < 0.0001$). There were differences between the reclaimed and native sites; the native sites exhibited more variability (although still significantly correlated), probably due to either differences in the relative lability of the substrates present or differences in the structure of the microbial communities present in the native versus reclaimed soils. We believe this method is of use as a relatively fast, accurate, and economical means by which soil quality can be ascertained. Estimates of SOC required to sustain nutrient cycling appears to be in the upper range of 0.1-0.7% C.

Additional Key Words: reclamation, semi-arid climate, microbial activity

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Introduction

Reclamation, while an extremely complex and involved process, has historically been more of an art than a science. There have, however, been considerable improvements in applying a more rigorous scientific methodology to this process in the recent past, and great strides have been achieved in maximising reclamation success. Many of these advances have been covered in a recent review article by Schuman (2002). However, the bulk of research undertaken has been concerned with maximising vegetation establishment, production, and to a lesser extent, biodiversity. While the interaction between the plant and soil communities is obviously complex, the ecological direction in which the soil ecosystem is moving will have strong implications for the development of the plant community and succession. Furthermore, the microbial communities present in soil play an extremely important role in ensuring, via the decomposition of soil organic matter (SOM), that nutrient cycling will provide sufficient nutrients to maintain a productive and stable plant community. As a result, indices of soil quality or health, and thus tools to measure these indices, need to be developed and incorporated into the reclamation process.

Measuring "soil health or quality" remains an extremely difficult and inexact process. This stems from the fact that, while theoretical definitions of soil health exist (Doran and Parkin, 1994), there is often little connection with measurable soil parameters. Soil quality indices are further complicated due to spatial and temporal variability as well as methodology concerns. In spite of these problems, it is critical that we obtain a better understanding of these soil indices due to their critical importance in maintaining a stable and permanent plant community. Criteria put forward by Doran and Parkin (1994) suggested that soil quality indicators should meet the following criteria:

1. Encompass ecosystem processes and be related to process orientated modelling,
2. Integrate soil physical, chemical and biological properties and processes,
3. Be accessible to many users and applicable to field conditions,
4. Be sensitive to variations in management and climate, and
5. Where possible, be components of existing soil data bases.

A paper published by Franzluebbers et al. (2000) put forward a method that seemed to encompass many of the criteria listed above. In that study, generally strong correlations were found between the flush of microbial respiration measured three days after rewetting a soil ('three-day CO_2 flush method'), and various other parameters that are commonly considered to be indicators of soil health and are also intimately linked to many of the previously listed criteria. These parameters included longer-term microbial respiration (24 days), microbial biomass, N-mineralization, organic C, and particulate organic matter. Franzluebbers et al. (2000) also observed regional and management differences in the correlations between three day microbial respiration and 24-day microbial respiration, microbial biomass, nitrogen N-mineralization and SOC. The three-day CO_2 flush method also has the advantages of being relatively fast, economical, and provides an apparently reliable way to estimate soil quality.

However, the research undertaken by Franzluebbers and colleagues (2000) utilised cropland soils from climatic areas that are often very different from the climate and soils found in the Powder River Basin of Wyoming. We wished to examine whether the 'three-day CO_2 flush method' could be used to aid in defining soil quality in semi-arid, drastically disturbed soils found in the reclaimed open-cut coal mine soils. We were also interested as to what level of SOC was necessary to ensure that adequate nutrient cycling occurred to support a sustainable and desirable plant community. We hoped to be able to estimate ('back of the envelope') the amount of SOC required to provide sufficient inorganic N to maintain the reclaimed plant community. Throughout this paper we will use soil SOC as a proxy for SOM as; we are able to much more accurately measure SOC; it does not require a correction factor (which is known to vary both betweens soil types as well as depth; Nelson and Sommers, 1996); and SOC is a more direct measure of SOM; and the data can be compared across climatic regions and soils at national and international scales.

Materials and Methods

The open-cut coal mines we sampled are located in the Powder River Basin of northeastern Wyoming, USA. This area typically has an annual mean precipitation of 392 mm, of which about 30% comes from snowmelt. July, the hottest month, has a mean maximum summer time temperature of 30.4^{o}C, and the mean minimum in January, the coldest month, is -11.8^{o}C. The mines are approximately 1,400 m above sea-level. All of the mines are currently operational, and the sites we sampled had all been reclaimed using standard reclamation techniques and similar species seed mixes. Seed mixes used varied from site to site, but included shrubs (Wyoming big sagebrush, *Artemisia tridentatat.* ssp. *wyomingensis*; fourwing saltbush, *Atriplex canescens*; rubber rabbitbrush, *Ericameria nauseosa*; winterfat, *Eurotia lanata*), C_3 species (western wheatgrass, *Pascopyrum smithii*; slender wheatgrass, *Elymus trachycaulus*; needleandthread, *Stipa comata*), and C_4 species (blue grama, *Bouteloua gracilis*). The native prairie sites are classified as northern mixed-grass communities and represent the single largest ecosystems of native grasslands remaining in the USA.

In the summer of 2000, a preliminary experiment was undertaken to examine whether the correlations that Franzluebbers et al. (2000) had observed between three-day microbial respiration measurements and other indicators of soil health and nutrient cycling in cropland soils would also hold true in disturbed reclaimed mine soils in a semi-arid environment. Permanent sampling sites were established at two different locations on each of four mines. These sites varied in topsoil handling treatments (direct-hauled or stockpiled), reclamation age and SOC concentrations (Table 1). Along a 40 m transect, soils were sampled at 10 m intervals in depth increments of 0-2.5, 2.5-15, and 15-30 cm. Soils were then taken to the laboratory, air-dried, and sieved (<2mm) before analysis. Total C was determined using dry combustion methods (Nelson and Sommers, 1996) on a Carlo-Erba C and N analyser, after roller-grinding the soils overnight. On the same roller-ground samples, inorganic C was measured using a modified pressure-calcimeter method (Sherrod et al., 2002), and organic C was calculated by subtracting inorganic C from total C. Total N was also determined by dry combustion. Soils were first dried for 48 hours at $55^{o}C$ and then rewetted to a soil water content of -0.05 MPa. Using approximately 50-60 g of soil, microbial respiration was measured using standard base trap methods (Zibilske, 1994) at 3 and 21 days. On a similar amount of soil, microbial biomass was calculated using the chloroform incubation method (Horwath and Paul, 1994) and a Kc of 0.41 (Kc is the fraction of microbial biomass C decomposed and released as CO_2-C in 10 days; Anderson and Domsch, 1978). Potential N-mineralization was determined on the same soil on which 21 day microbial respiration was measured by calculating the difference in inorganic N (N_i; NO_3 + NH_4) between day 21 and day 0. Inorganic N was measured on a TRAACS Auto-Analyser after extracting with 1 M KCl. Regression analyses were calculated (Statview v. 4.51, Abacus Concepts, Inc. 1995) between three-day microbial respiration and each of the other microbial and soil parameters evaluated.

Table 1. Combined biological, organic C, and total N for various reclaimed and native sites data from 2000 and 2001 sampling.

Site	Depth (cm)	Organic C (%)	Total N (%)	3-day Microbial respiration (mg C kg soil^{-1})	21-day Microbial respiration (mg C kg soil^{-1})	Microbial biomass (mg C kg soil^{-1})	Nitrogen-mineralization (mg N_i kg soil^{-1})
NA85[#]	0-2.5	4.37	0.214	346	883	1,202	45
	2.5-15	0.84	0.049	73	165	279	8
NA97	0-2.5	2.61	0.111	197	494	736	13
	2.5-15	0.98	0.067	83	199	308	3
NA-Prairie	0-2.5	1.37	0.109	135	368	561	13
	2.5-15	0.72	0.075	57	152	239	4
BA55	0-2.5	1.96	0.140	177	460	842	25
	2.5-15	0.78	0.080	70	174	308	8
BA72	0-2.5	1.30	0.096	131	340	502	17
	2.5-15	0.78	0.066	67	146	207	5
BA-Prairie	0-2.5	1.46	0.135	157	468	846	19
	2.5-15	0.90	0.092	79	216	353	7
JRSP	0-2.5	0.99	0.074	149	488	568	10
	2.5-15	0.84	0.061	77	204	247	4
JRDH	0-2.5	0.58	0.051	82	231	222	8
	2.5-15	0.45	0.043	33	75	96	1
JR-Prairie	0-2.5	2.86	0.160	140	479	667	26
	2.5-15	1.03	0.109	64	208	289	16

[#]NA-North Antelope Coal mine, Peabody Energy; BA-Belle Ayr mine, RAG Coal West, Inc.; JR-Jacobs Ranch mine, Kennecott Energy. All of these mines are southeast of Gillette, WY.

As a result of the promising correlations that we observed from the results obtained from the 2000 samples, in the summer of 2001 we returned to three of the original mines and established three, 40 m transects at the original samples sites. We also sampled a control site; an undisturbed, native prairie site that was situated within a few kilometres of the reclaimed sites and were representative of the reclaimed soils. Control site sampling was undertaken in exactly the same manner as the reclaimed sites by laying out three, 40 m transects, that were sampled at 10 m intervals using depth increments of 0-2.5 and 2.5-15 cm. The 15-30 cm depth wasn't sampled because our previous results indicated that the biological activity was concentrated in the surface 15 cm of the soil. Soils were air-dried and sieved (<2mm) and the same analyses as noted above were repeated. Regression analysis was undertaken using the samples from both years (n = 366).

On the same transects on which the soils where sampled, at the 0 and 40 m interval we sampled the vegetation, and dried (55^{o}C) it to obtain an estimate of biomass. A sub-sample was taken and roller-ground overnight and analysed for C and N and allowed us to obtain an estimate of the plant communities annual N uptake.

Results and Discussion

The three-day microbial respiration data were significantly correlated with all of the parameters measured (Fig. 1). There were apparent differences in the correlation coefficients between the three-day microbial respiration and other indicators of soil quality (i.e. 21-day microbial respiration, microbial biomass, N-mineralization, organic C and N) between reclaimed and native soils (Table 2), although both were highly significant. In reclaimed soils, correlations were generally much less variable than in native soils (Table 2) suggesting that in these disturbed ecosystems the microbial populations were much more responsive to changes in specific soil environmental characteristics (e.g., organic C and N). Moreover, as litter (both above-, and below-ground) present on the reclaimed mine sites is relatively new (<16 years), the vast majority of this material (either as soluble organic C or particulate organic matter) is likely to be readily available for decomposition and mineralization. On the other hand, it is likely that a large proportion of the C present in SOM will be of a recalcitrant form and thus less available for microbial activity. In addition, soils used in the reclamation process are much more homogeneous (due to the topsoil salvage, mixing, replacing, and seedbed preparation processes) compared to the spatially more heterogeneous native, undisturbed prairie ecosystem. This also will have a considerable bearing on the variability observed in the regression analysis between the reclaimed and native, prairie sites. This disparity between the responses of the reclaimed and native microbial populations may be the result of differences either in the relative availability of C and N in the two soil types (with potentially a large labile pool of C and N occurring in the reclaimed soils) or differences in the structure and makeup of the microbial communities present in the reclaimed soils verus native soils.

Table 2. Comparison of regressions between native and reclaimed soils

3 day MR* vs.	Treatment	Regression equation	r^2	P-value
21 day MR*	Native	$y = 2.712x + 29.518$	0.81	< 0.001
	Reclaimed	$y = 2.649x – 10.370$	0.94	< 0.001
Microbial biomass	Native	$y = 4.893x – 7.824$	0.88	< 0.001
	Reclaimed	$y = 3.836x – 5.606$	0.83	< 0.001
N-mineralization	Native	$y = -1.977 \text{ x } 10^{-4}x^2 + 0.182x - 2.292$	0.50	< 0.001
	Reclaimed	$y = -7.813 \text{ x } 10^{-5}x^2 + 0.138x – 4.938$	0.61	< 0.001
Organic C	Native	$y = -1..422 \text{ x } 10^{-5}x^2 + 0.014x – 0.127$	0.47	< 0.001
	Reclaimed	$y = 1.863 \text{ x } 10^{-45}x^2 + 0.005x - 0.280$	0.80	< 0.001
Total N	Native	$y = 1.209 \text{ x } 10^{-6}x^2 + 2.669 \text{ x } 10^{-4}x – 0.070$	0.63	< 0.001
	Reclaimed	$y = 3.553 \text{ x } 10^{-7}x^2 + 3.782 \text{ x } 10^{-4}x – 0.028$	0.69	< 0.001

*Microbial respiration

Drying and sieving of the soil undoubtedly had an impact on measured values of microbial activity (i.e., microbial biomass and respiration). However, previous work has found little difference between estimates of soil microbial biomass regardless of whether biomass measurements were taken on either field moist soils, or soils that where dried and pre-incubated for ten days before biomass measurements were undertaken (Franzleubbers et al., 1996, Franzluebbers, 1999). The authors would like to point out that the aim of this work is not to obtain *absolute* values of the biological parameters of interest but rather to obtain *comparative* values of soil biological properties - which are indicative of the ability of any particular soil to sustain nutrient cycling and thus supply a sufficient quantity of nutrients to maintain plant growth and production. In addition, we are more interested in the biological activity of soil (rather than physical properties) because microbes are responsible for all of the important processes (i.e., decomposition, mineralization) by which nutrients are made available for plant uptake.

As mentioned previously, one objective of this research was to establish a “rule of thumb” estimate as to the amount of SOC that would be required to maintain an adequate level of nutrient cycling in these low N environments. To obtain this SOC estimate we extrapolated the N-mineralization data to a field situation (by assuming that the rate of N-mineralization was observed in the lab will be comparable to that found in the field, and, will occur over the entire course of the defined growing period) using two different scenarios: a growing period that extends from the 1st May – 31st June, or a longer growing period, 1st May – 31st August. The results of this exercise indicate that *potentially*, there is more than sufficient mineralization of organic N to satisfy the requirements of plant communities on these reclaimed mine sites (Table 3), regardless of which of the two growing periods we use. A study undertaken by Woods and Schuman (1986) in a nutrient and organic matter poor, reclaimed mine site, examined relationships between SOC (measured by the Walkley-Black method) and microbial biomass, N-mineralization, and plant N. They estimated that somewhere between 0.1-0.7% SOC would be required to maintain nutrient cycling in this particular ecosystem. Our results indicate that the concentration of SOC necessary to ensure adequate nutrient cycling may be nearer the upper end of the range they predicted.

Table 3. Aboveground plant biomass, N content, and potentially available N-mineralized for two different growing season.

Site	Aboveground plant biomass (kg ha^{-1})	Aboveground plant N content (kg N ha^{-1})	*Potential* N-mineralized* (kg N ha^{-1}) 1st May - 31st June	*Potential* N-mineralized* (kg N ha^{-1}) 1st May - 31st August
NA85#	815	16	101	203
NA97	1,140	15	36	72
NA-Native	488	6	40	80
BA55	978	14	85	171
BA72	567	15	44	89
BA-Native	590	8	69	139
JRDH	1,289	18	21	42
JRSP	1,231	15	49	98
JR-Native	561	8	143	288

* Calculated by assuming that that in the period between the 1st May - 31st June or 1st May - 31st August that N will be mineralized at the same rate as determined in the 21-day N-mineralization laboratory experiment.

NA-North Antelope Coal Mine, Peabody Energy; BA-Belle Ayr Mine, RAG Coal West, Inc.; JR-Jacobs Ranch Mine, Kennecott Energy. All of these mines are southeast of Gillette, WY.

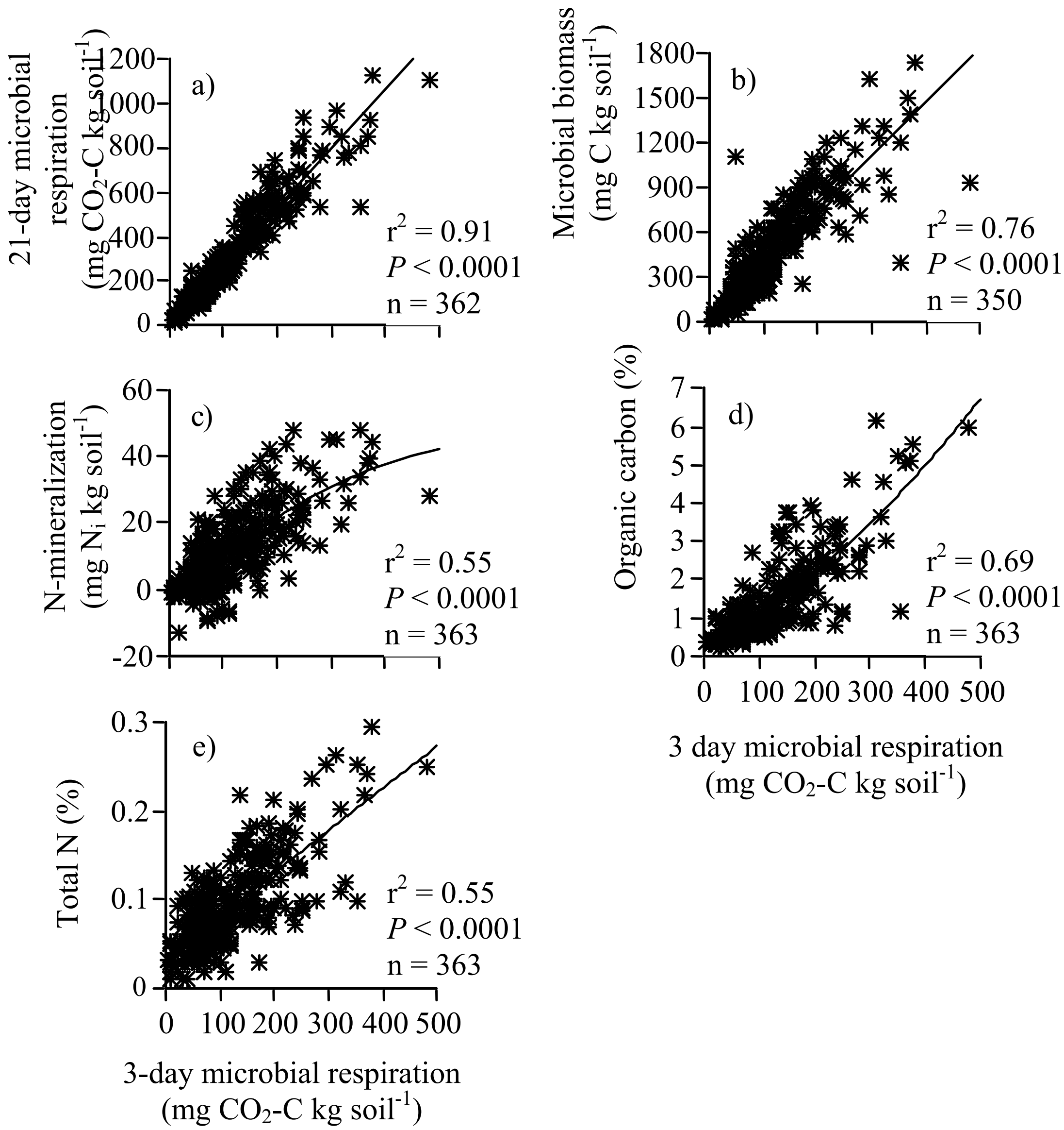

Figure 1. Regressions (reclaimed and native soils combined) of 3-day microbial respiration against: a) 21-day microbial respiration; b) microbial biomass; c) potential N-mineralization (a negative value indicates net N-immobilization); d) organic C, and; e) total N.

In our research we have measured SOC values of up to 6% (Fig. 1d), which is clearly an unrealistic value for these semi-arid ecosystems and reflects the presence of particulate coal material. We are unable to differentiate between the mineralizable fraction of SOC (i.e. SOM) and the organic C that is essentially unmineralizable (i.e., coal). Nonetheless, although our values for SOC are clearly high, it may not be a major concern because where SOC was high, microbial activity was also elevated (Table 1). However, this may not be true in all situations and for all reclamation sites. More importantly, we will need to ensure that, in our assessment of a minimum level of SOC, coal dust is not a major component of SOC values.

In conclusion, we would like to point out that we are not proposing that we have devised the final indicator of soil quality and reclamation success. However, from the generally strong correlations we observed between three-day microbial respiration and other commonly accepted and standardised parameters of soil health/quality, we feel that this method provides those involved with mine reclamation a tool to maximise the limited soil resource and ensure SOC quality control in topsoil salvage and replacement. At this time we are unable to predict quantitatively the other soil health/nutrient cycling indicators on the basis of three-day microbial respiration alone, but we can on a relative basis rank the "health" of soils used in the reclamation process. As such this method will give those involved with reclamation the ability to categorise the quality of soils as it relates to the cycling of nutrients and SOM, and thereby optimise the limited soil resources in order to maximise plant production and the potential for sustainable and successful reclamation.

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