MICROBIAL AND SOIL ENZYME ACTIVITIES IN STOCKPILED TOPSOIL AND COAL MINE SPOIL MATERIALS[1]

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Abstract.--Dehydrogenase, nitrogenase, urease, phosphatase, arylsulphatase, amylase, cellulase, invertase and protease activities of stockpiled topsoil and coal mine spoil overburden material were determined, and compared to an undisturbed reference soil. The undisturbed soil had higher enzyme activities than in the stockpiled topsoil or spoil materials. With the exception of protease, the spoil material had the lowest enzyme activities. The low enzymatic activities found in the stockpiled topsoil, and especially in the spoil material may be partially attributed to the poor chemical and physical environment of these materials, as well as to the low microbial population and diversity of microorganisms.

INTRODUCTION

In the process of surface mining, topsoil is removed and stockpiled, and is often held for an extended time before it is respread and revegetated. During topsoil storage, many of the attributes of undisturbed soils are modified, including the disruption of soil structure and an increase in soil bulk density. Less obvious is the loss of organic matter and the disruption of normal biological processes.

Several researchers have reviewed and investigated the microbial problems associated with topsoil storage (Cundell 1977; Miller 1978; Miller and Cameron 1976; Miller et al. 1976; Rives et al. 1980; Fresquez and Lindemann 1982; Fresquez and Aldon 1984). They found that stockpiling of topsoil reduced the number of microbes and mycorrhizal spores, and the diversity of fungi, algae, and protozoa. Also, the numbers of microorganisms and fungal diversity are known to be lower in spoils derived from overburden material than in either stockpiled topsoil or undisturbed soil (Wilson 1965; Fresquez and Lindemann, 1982; Fresquez and Aldon 1984).

The lower microbial population, and especially the low diversity of microorganisms observed in spoil and stockpiled topsoil may be related to decreased enzymatic capabilities. Such reduced enzymatic capabilities may hinder normal nutrient cycling and decomposition processes (Fresquez and Lindemann 1982). The objective of this study was to determine the biogeochemical quality of stockpiled topsoil and coal mine spoil overburden materials from a coal surface mine in northwestern New Mexico, and relate these values to those of an adjacent undisturbed soil.

MATERIALS AND METHODS

Soils

The soil and spoil samples used in this study were from the San Juan coal surface mine near Farmington, N. Mex. Soils, vegetation, and environmental parameters of this area were described previously (Fresquez and Lindemann 1983).

Five composite soil samples were collected randomly from a spoil overburden pile that was 2 to 3 years old, a topsoil stockpile that was 3 to 4 years old, and an adjacent undisturbed area. Each composite sample was derived from 10 subsamples that were collected randomly to a depth of 13 cm, using a hand probe. The 10 subsamples were mixed thoroughly in a clean plastic bag, transferred to a sterile bag, and immediately cooled in an ice chest for transport to the laboratory. At the laboratory, the composite samples were passed through a 2-mm sieve, and were stored at 4°C before the microbial and soil enzyme analyses. A composite sample from each material was submitted for chemical analysis at the New Mexico State University Soil and Water Testing Laboratory. All methods of analysis have been described previously

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(Fresquez and Lindemann 1983). The chemical and physical properties of the spoil and soil materials are given in (table 1).

Biochemical Analysis

All microbial and soil enzyme measurements were completed within 9 weeks in the following order: dehydrogenase, nitrogenase, urease, invertase, amylase, phosphatase, cellulase, arylsulphatase, and protease. Dehydrogenase activity was measured by a modification of the procedure of Skujins (1973). To allow for more complete mixing and soil saturation in the high clay content spoil materials, 3.5 ml of water was added to 6 g of soil (Fresquez and Lindemann 1982), for the dehydrogenase assay. Formazan production was compared to a standard curve using triphenyltetrazolumformazan in methanal. Values were measured on a Basch and Lomb spectrophotometer 21, and are reported as micrograms of formazan per gram of oven-dry soil per day.

Nitrogenase activity was assayed by the acetylene reduction technique, using a modification of the procedure of Stewart et al. (1967). Duplicate 2-g soil samples were placed in 10 ml (13 x 60 mm) serum stopper sealed containers, moistened with 1 ml of a 0.5% glucose solution, and incubated at 23°C in the dark under an atmosphere of nitrogen gas and 0.6 ml of acetylene for 24 hours. The head space of the serum bottles was analyzed for ethylene by injecting a 0.2 ml gas sample into a Hewlett-Packard 5750 gas chromatograph. Nitrogen was used as the carrier gas, with a flow rate of 25 ml per minute through a 2.74 m long stainless steel column packed with 100 to 200 mesh Porapak T, using column, injector, and detector temperatures of 100° C. Acetylene reduction activity was calculated by comparing ethylene production to known ethylene standards on a Hewlett-Packard 1040 integrator set at an attenuation of 1. Results are reported as nm C_2H_4 per gram of oven-dry soil per day.

Extracellular hydrolytic enzyme assays were carried out using a Beckman Acta CIII UV-Visible spectrophotometer, and are expressed as micrograms of product per gram of oven-dry soil per day. Urease activity was determined by the general method of Pancholy and Rice (1973), except that a 2.5 M KCl-Ag_2SO_4 solution (Tabatabai and Bremner 1972) was used, and the amount of evolved ammonia was measured by Nesseler's method described by Allen (1957). Sigma ammonia color reagent (Sigma Chemical Company) was used instead of Nessler's reagent. Cellulase activity was assayed by the method described by Pancholy and Rice (1973). Invertase and amylase activity was measured by the method of Ross (1965), with the reducing sugar of the filtrate determined by Nelson's method (Clark 1969). Phosphatase activity was measured using the p-nitrophenol phosphate substrate and method of Tabatabai and Bremner (1969). Arylsulphatase activity was determined by the method of Tabatabai and Bremner (1970) using p-nitrophenyl sulphate as a substrate. Protease activity was measured by the method of Speir and Ross (1975).

Statistical Analysis

For all measurements except nitrogenase, urease, and protease, significant differences ($p \leq 0.05$) between sites were determined using analysis of variance and Tukey's Multiple Range Test. Bartlett's test for homogeneity of variances showed that nitrogenase, urease, and protease had heterogeneous variances. Therefore, Dunnett's Pairwise Multiple Comparison Test (Dunnett 1980) for means in the unequal variance cases was used to determine significant differences in the values of nitrogenase, urease and protease between sites at the 5% probability level.

RESULTS

Soil differences

Major differences were found in chemical and physical properties between the undisturbed soil and the disturbed soil and spoil materials (table 1). The stockpiled topsoil, and especially the spoil overburden material generally had higher EC, SAR, Ca, Mg, Na, K, SO_4, NO_3-N, total N, organic matter, CEC, and clay contents than did the undisturbed soil. Although the spoil material contained 1120 ug g^{-1} total N and 435 g kg^{-1} organic matter, the nitrogen and carbon in these materials includes coal and other humic materials that may not be readily mineralized or oxidized by microorganisms (Reeder and Berg 1977; Fresquez and Lindemann 1982). Soil pH and especially P were higher in the undisturbed soil than in the stockpiled topsoil or spoil materials.

Biochemical differences

There were significant differences among the undisturbed soil and disturbed soil and spoil materials for all enzyme assays, except for cellulase (Table 2 and 3). In general, enzyme activity was higher in the undisturbed reference soil than in the stockpiled topsoil or the spoil overburden materials. Most enzymes, particularly dehydrogenase, nitrogenase, amylase and protease were lower in stockpiled topsoil than in the undisturbed soil. With the exception of protease, the spoil material had the lowest enzymatic activity.

DISCUSSION

Stockpiled topsoil and spoil overburden materials are characterized by having low microbial populations, as well as a low diversity of microorganisms. For example, Fresquez and Aldon (1984), reported that the diversity of fungal genera in these same disturbed soil and spoil materials were considerably less than the diversity in the undisturbed reference soil. The stockpiled topsoil material, in which 11 fungal genera were identified, had a higher diversity index than the spoil material. Only four fungal genera were representative of the spoil material, with Penicillium and Chrysosporium comprising 88% of the total colonies isolated.

Table 1--Characteristics of an undisturbed soil, stockpiled topsoil and spoils materials from the San Juan coal surfacemine.

Soil parameter[1]	Undisturbed soil	Stockpiled topsoil	Overburden spoil
EC[2]	0.56	2.64	9.72
SAR	1.12	6.75	27.58
pH	7.83	7.72	7.13
Ca	89.15	182.58	345.78
Mg	11.55	34.66	45.72
Na	42.09	376.97	2041.25
K	23.46	32.06	52.00
P	1.68	1.10	0.69
SO_4	0.73	34.35	100.77
NH_4	3.40	2.40	4.40
NO_3	0.10	0.10	10.10
Total N	247	305	1120
Organic Matter	5.4	6.4	43.5
CEC	3.14	4.06	15.10
Texture	Loamy sand	Sandy loam	Clay

[1]Water-soluble cations, pH, EC and SO_4 determined from saturated paste extract. Soil P, NH_4-N determined from 1:5 water extract. Organic matter determined by the Walkley-Black method, and total N determined by the macro-Kjeldahl method.

[2]EC is expressed as dS m^{-1}, Ca, Mg, Na, K, P, and SO_4, NH_4, NO_3, and total N are expressed as ug g^{-1}, organic matter is expressed as g kg^{-1}, and CEC is expressed as cmoL(+) kg^{-1}.

Table 2--Microbial and soil enzyme activities of an undisturbed soil, stockpiled topsoil and spoil materials from the San Juan Coal surfacemine.

Site	Dehydrogenase	Nitrogenase	Urease	Phosphatase	Arylsulphatase
	ug Formazan $g^{-1}d^{-1}$	nm C_2H_4 $g^{-1}d^{-1}$	ug NH_4-N $g^{-1}d^{-1}$	ug PNP $g^{-1}d^{-1}$	
Undisturbed soil	10 a[1]	204 a[2]	834 a	368 a	30 a
Stockpiled topsoil	3 b	21 b	563 a	304 a	32 a
Overburden spoil	2 b	6 b	205 b	140 b	1 b

[1]Dehydrogenase, phosphatase and arylsulphatase means within the same column followed by the same letter are not significantly different at the 0.05 level by Tukey's Multiple Range test.

[2]Nitrogenase and urease means within the column followed by the same letter are not significantly different at the 0.05 level by Dunnett's Pairwise Multiple Comparisons Test.

Table 3--Soil enyzme activities of an undisturbed soil, stockpiled topsoil and spoil materials from the San Juan coal surfacemine.

Site	Amylase	Cellulase	Invertase	Protease
	ug glucose $g^{-1}d^{-1}$			ug tyrosine $g^{-1}d^{-1}$
Undisturbed soil	131 a[1]	52 a	1225 a	249 ab[2]
Stockpiled topsoil	75 b	35 a	1184 a	116 b
Overburden spoil	5 c	23 a	272 b	681 a

[1]Amylase, cellulase and invertase means within the same column followed by the same letter are not significantly different at the 0.05 level by Tukey's Multiple Range Test.

[2]Protease means within the same column followed by the same letter are not significantly different at the 0.05 level by Dunnett's Pairwise Multiple Comparisons Test.

Generally, a decreased microbial diversity often characterizes extreme physiochemically stressed and unstable soil environments (Atlas and Bartha 1981). The lower microbial populations and a microbial diversity, observed in these stockpiled topsoil and spoil materials, may have been partially responsible for the reduced enzymatic activities in these materials. Of the parameters tested, dehydrogenase, nitrogenase, amylase, and protease were most affected by topsoil storage. The activity of most enzymes, with the exception of cellulase and protease, were significantly decreased in the spoil material.

The reduction of various microbial and soil enzyme activities by stockpiled topsoil and spoil material compared to the undisturbed reference soil was shown in this study, although the exact causes of enzyme inhibition was not. The soil chemical and physical property data do not provide statistical information helpful for interpretating the decreased enzyme activities observed in these spoil and soil materials. However, indications are that many physiochemical properties, in stockpiled topsoil, and especially in spoil material, were able to influence and consequently reduce or prevent the development of many of these biological processes. Chemical and physical soil properties, particularly salinity and high clay contents, may markedly influence enzyme activity. Stroo and Jencks (1982), for example, reported that amylase decreased with increasing clay content. Dehydrogenase, and to a lesser extent the hydrolases, decreased with increasing EC (Frankenberger and Bingham 1982). Pancholy and Rice (1973) found no correlation between enzymatic activity, particularly amylase, cellulase, and invertase and the soil organic matter content or pH. Specific toxic components may also influence soil enzymatic processes. Klein et al. (1985) provided a summary of information on other components which might influence enzyme activities, particularly toxic inorganic (Ag, As, B, Cd, Hg, Mo, Se, F), or organic compounds (polynuclear aromatic hydrocarbons; carbazole, acridine, anthracine and phenanthrene) that may reduce soil enzymatic activity in coal mine spoils.

The reduction of various microbial and soil enzymes activities in the stockpiled topsoil and spoil material, when compared to the undisturbed reference soil, are in general agreement with the results of other investigators. Dehydrogenase, nitrogenase, amylase, urease and phosphatase activities have been found to be lower in spoil material than in undisturbed soils (Stroo and Jencks 1982; Visser et al. 1983 Hersman and Klein 1979; Sörensen et al. 1981; Miller 1978; Barth 1984). With the exception of Miller (1978) and Fresquez and Lindemann (1982) who found that protolytic and dehydrogenase activity were reduced in stockpiled topsoil compared to an undisturbed soil, no other data concerning the effects of topsoil storage on these enzymatic activities were found in the literature.

In conclusion, these baseline data indicate that stockpiled topsoil, and especially spoil overburden material are characterized by extremely adverse chemical and physical soil properties compared to the undisturbed soil. Adverse stored soil and spoil properties may reduce the of number microbes (Fresquez and Lindemann 1982) and decrease the diversity of fungal genera (Fresquez and Aldon 1984). Although the enzyme activities in stockpiled topsoil were decreased in comparison with the undisturbed soil, the two were more similar in enzyme activities than they were to the spoil overburden material. The spoil material had the lowest enzyme activities. Covering spoil materials with the biologically more active stored topsoil will insure a more rapid reestablishment of nutrient cycles and decomposition processes in reclaimed minelands.

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