DIVERSITY AND COMPOSITION OF SOIL FUNGI ASSOCIATED WITH RECLAIMED COAL MINE SPOILS AND SOILS[1]

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Abstract.--The soil fungal community associated with an undisturbed soil was described and compared with the fungal communities from spoil overburden and stockpiled topsoil materials, and from eight reclaimed topsoiled and nontopsoiled areas of varying ages. The diversity and evenness of fungal genera were higher in the undisturbed soil than in any of the disturbed overburden spoil or stockpiled topsoil materials, or in any of the reclaimed topsoiled and nontopsoiled areas. Stockpiled topsoil had a fungal community with a higher diversity and more similarity to the undisturbed soil than did the spoil overburden material. When the reclaimed areas were compared, the topsoiled areas had a higher diversity and a fungal community more closely related to the undisturbed soil than did any of the nontopsoiled areas. The composition of fungal genera was related to soil properties and site age. In the topsoiled areas, Penicillium dominated the youngest topsoiled site, while Aspergillus dominated the oldest topsoiled site. In contrast, Aspergillus dominated the youngest nontopsoiled site, while Penicillium dominated the oldest nontopsoiled area.

INTRODUCTION

Several investigators have used microfungal population estimates, and particularly fungal diversity and community composition responses, to gain a better understanding of ecosystem functioning and development on disturbed areas. Wilson (1965) was the first to characterize fungal populations associated with coal mine spoil overburden materials, and to show that populations were markedly reduced in nonvegetated spoil areas as compared to revegetated spoils or to undisturbed areas. More recently, Lawrey (1977) reported that spoil material had lower fungal diversity than undisturbed soil, and that the low diversity could have been due to the absence of organic matter in the form of litter. Fresquez and Lindemann (1982) reported similar results.

Visser et al. (1983) described the fungal composition associated with an undisturbed soil and a coal mine spoil in western Canada. The fungal community shifted from one dominated by Chrysosporuim/Pseudogymnoascus/Sterile dark forms in the undisturbed soil to one dominated by Alternaria/Cladosporium/Sterile dark forms/Yeasts in the spoil overburden materials. Allen and MacMahon (1985) contrasted the fungal communities and ecosystem properties of two adjacent sites, an undisturbed shrub-steppe and a strip-mined site, immediately after topsoil replacement. They hypothesized that microbial populations and even diversity may or may not be seriously impacted during disturbance and soil replacement; however, the structure or the community composition would always be changed. They concluded that ecosystem components in disturbed areas may best be understood by rating their explicit spatial patterns and the community composition (an organism-centered approach).

The composition of soil fungi is related to the properties of the soil, as well as to the type of vegetative cover during succession (England and Rice, 1957; Brown, 1958; Wohlrab et al., 1963; Mallik and Rice, 1966). To date, only limited observations concerning the changes in the fungal community during plant establisment on coal mine spoils have been reported (McMullen and Stack, 1984; Otroaina et al., 1984). In this investigation, an attempt was made to determine the population size, diversity, and community composition of soil fungi associated with spoil overburden and stockpiled topsoil materials, and

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to describe the changes in the community structure as a result of topsoiling and revegetation over time.

MATERIALS AND METHODS

The soil and spoil materials used in this study were from the San Juan coal strip-mine near Farmington, New Mexico. Soil samples were collected from a spoil overburden pile (approximately 2 to 3 years old), a topsoil stockpile (approximately 3 to 4 years old), and an adjacent undisturbed soil. Also, samples of soil were collected from eight reclaimed sites. The reclaimed sites varied in age, soil characteristics, vegetation, and type of post-mining revegetation treatment. Three of the older disturbed sites (1974, 1975, and 1976) were reclaimed by grading and leveling the overburden spoil material (no topsoil was applied); mulching with 4.5 metric tons of grass hay ha^{-1} crimped 15 cm deep; fertilizing with 72 kg N and 90 kg P ha^{-1}; seeding with four native plant species (fourwing saltbush (Atriplex canescens), galleta (Hilaria jamesii), alkali sacaton (Sporobolus airoides) and Indian ricegrass (Oryzopsis hymenoides)); and irrigating for two growing seasons. The younger disturbed sites (1978, 1979, 1980, 1981, and 1982) were reclaimed using the same amendment materials and revegetation techniques as in the older sites, but differed in that stockpiled topsoil material was placed 20 to 30 cm deep over the recontoured spoil. Ten plant species were seeded in the newer sites; the same four plant species as in the older sites, plus sand dropseed (Sporobolus cryptandrus), western wheatgrass (Agropyron smithii), streambank wheatgrass (Agropyron riparium), winterfat (Eurotia lanata), blue grama (Bouteloua gracilis), and shadscale (Atriplex confertifolia)).

In July of 1982, five soil samples were collected from each site. Each sample consisted of 10 composited subsamples collected randomly to a depth of 13 cm with a hand probe. The 10 subsamples were mixed thoroughly in a sterile plastic bag and immediately cooled in an ice-chest for transport to the laboratory. At the laboratory, the samples were passed through a 2-mm sieve and stored at 4°C before fungal enumeration. An aliquot from each composited site sample was taken for laboratory analysis at the New Mexico State University Soil and Water Testing Laboratoy (Table 1).

Numbers of fungi were estimated by the dilution and plating technique of Wollum (1982). For population estimates, dilutions were plated in triplicate on rose bengal-streptomycin agar (Martin 1950) and incubated at 30°C for 4 days. The number of fungi were reported on a per gram of oven-dry soil basis. Fungal groups were isolated by evenly distributing 1 ml of a 10^3 dilution over the surface of solidified rose bengal-streptomycin agar. Six plates were inoculated for each composited site sample, then incubated at 30°C for 7 days. Fungi were classified by genus using the taxonomic guides of Barnett and Hunter (1972), Barron (1968), and Gilman (1968).

Variations in fungal populations between the undisturbed soil and the overburden spoil and stockpiled topsoil materials, and reclaimed nontopsoiled and topsoiled sites were analyzed using standard analysis of variance on natural log-transformed data. Tukey's multiple range test ($P < 0.05$) was used to compare population means of these organisms between the sites.

Four diversity indices were employed to compare the distribution pattern for each of the fungal genera isolated. First was Shannon's index of species diversity (Zar, 1974), which estimates community richness as

$$H = -\sum_{i=1}^{k} pi \log pi \qquad (1)$$

where pi = the proportion of genera i in the sample, and k = the number of genera. The corresponding test for evenness is

$$J = H/H_{max} \qquad (2)$$

where H_{max} = the maximum possible diversity.

An estimate of the similarity in the genera composition among the sample populations was calculated with Sorensen's presence community coefficient (SPCC), which was described by Mueller-Dombois and Ellenberg (1974) as

$$SPCC = 200C/(A + B) \qquad (3)$$

where C is the total number of genera common to two samples, A is the total number of genera in Sample A, and B is the total number of genera in Sample B. If the same genera were found in both samples, then the community coefficient would be 100, whereas if they had no genera in common, the coefficient would be 0.

Sorensen's quantitative community coefficient (SQCC), which estimates the similarity in the relative abundance of genera between samples, was also calculated. The SQCC is described by Mueller-Dombois and Ellenberg (1974) as

$$SQCC = 200Mw/(M_A + M_B) \qquad (4)$$

where Mw refers to the total of the smaller quantitative values of the genera common to two sample, M_A is the total number of individual isolates in Sample A, and M_B is the total number of individual isolates in Sample B.

RESULTS AND DISCUSSION

Numbers of fungal propagules were not significantly different in the undisturbed soil, the overburden material or the stockpiled topsoil

material (Table 2). A comparison between the reclaimed nontopsoiled and topsoiled areas with the undisturbed soil shows that the numbers of fungal propagules in the first reclamation year (1982) were significantly higher compared with the undisturbed soil. Populations of fungi increased in the year after topsoiling and revegetation (1981), and then decreased after aging four years (1978) to values comparable to those of the undisturbed soil. Numbers of fungal propagules in the older nontopsoiled spoil areas (1974, 1975, and 1976) were all significantly higher compared with the undisturbed soil.

The undisturbed soil was characterized by a higher fungal diversity (H) and evenness (J) index than either the overburden spoil or stockpiled topsoil materials (Table 3). The undisturbed soil was dominated by Curvularia, Penicillium, and an unidentified isolate. The stockpiled topsoil material, which was dominated by Penicillium and Aspergillus had a higher fungal diversity than the spoil overburden material. Only four fungal genera were dominant in the spoil material, with Penicillium and Chrysosporium comprising 88% of the total colonies isolated.

The diversity and evenness of fungal genera were also found to be higher in the undisturbed soil than in any of the reclaimed topsoiled or nontopsoiled areas. Considering all the reclaimed sites, the topsoiled sites exhibited higher diversity and evenness indices than any of the older nontopsoiled areas. The most recently reclaimed topsoiled site (1982) was dominated by Penicillium and Stachybotrys, and after four years (1978) shifted to an Aspergillus/Penicillium dominated fungal community. All three nontopsoiled spoil areas, depending on age, were dominated by either Aspergillus or Penicillium.

Sorensen's presence (SPCC) and quantitative (SQCC) community coefficients indicated that the fungal genera of the stockpiled topsoil material were more similar to those of the undisturbed soil than those of the overburden spoil material (Table 2). The types and quantity of fungal genera isolated in all of the topsoiled areas were more nearly similar to the undisturbed soil than were any of the older nontopsoiled areas.

Diversity indices can be used to express the degree of physiochemical stress placed on the soil biological community (Atlas and Bartha, 1981). Generally, a high diversity of microbial species, and few individual organisms per species are indicative of a stable and well-balanced soil ecosystem. In contrast, low microbial diversity with high numbers of individual organisms per species indicate an unstable or stressed soil ecosystem. This study showed that the undisturbed soil had a higher fungal diversity and evenness index than the overburden spoil and stockpiled topsoil materials, and the reclaimed areas. The spoil material had the lowest diversity of fungi. Adverse soil chemical and physical properties in spoil overburden materials may have reduced the diversity of fungi, a result possibly related to the high total salt and high total sodium, and high clay content (48%) of these materials. Although the total nitrogen and organic matter content of the spoil material were high, the nitrogen and carbon in these materials has been reported to be derived mainly from coal, humic materials, and other organic materials not readily mineralized or oxidized by microorganisms (Reeder and Berg, 1977; Fresquez and Lindemann, 1982). The data from the present study are in agreement with others that found the fungal taxa in spoil materials to be less diverse than those in undisturbed soils (Lawrey, 1977; Miller et al., 1979; Fresquez and Lindemann, 1982).

Although the stockpiling of topsoil material also resulted in lower diversity than the undisturbed soil, the placement of stockpiled topsoil material 20 to 30 cm deep over the recontoured spoil material resulted in significantly higher fungal propagule populations than observed in the undisturbed soil only 3 months after topsoiling and revegetation. In addition, the diversity of fungal genera was relatively high in comparison to the undisturbed soil. Penicillium and Stachybotrys dominated this newly topsoiled site (1982), demonstrating that Penicillium may be one of the first organisms to colonize disturbed areas. Visser et al. (1982) reported that Penicillium would probably be one of the first fungal groups to colonize disturbed areas because they are generally among the most common air-borne forms. Also, Penicillium can utilize a wide range of substrates, and can survive under extreme environmental conditions (Domsch et al., 1980).

The numbers of fungal propagules, the diversity of fungal genera and the composition of fungal groups changed with the advance of plant establishment in the topsoiled areas. The decline in fungal propagules probably reflected the exhaustion of added carbon and nitrogen from the hay and fertilizer amendment (Table 1), and decreased plant productivity after irrigation was terminated. In contrast to the declining fungal populations, the diversity of fungal genera generally increased with age up to the transitional stage (1979), but decreased slightly in the oldest topsoiled area (1978). The oldest topsoiled site (1978), however, still had a relatively high fungal diversity in comparison to the undisturbed soil. These data are in agreement with those of Mallik and Rice (1966), and conform in general with the principle of Odum (1963), in which the number of species of heterotrophs increase over time.

Many of the fungal genera isolated from the topsoiled areas were common to all areas. Reviews by Orpurt and Curtis (1957), Brown (1958), and Wohlrab et al. (1963) indicated that fungal species did not form discrete communities in which particular forms were constantly associated but appeared to be arranged in a continuum of the same type as that shown by the

Table 1. Chemical characteristics of an undisturbed soil, and disturbed and reclaimed coal mine spoils and soils.

Site	Water-soluble cations[1]				Phosphorus and nitrogen[2]				CEC	Organic matter[3]	EC	pH	SAR
	Na	Ca	Mg	K	P	NH_4-N	NO_3-N	TKN					
	meq L^{-1}				ppm				meq $100\ g^{-1}$	%	mmhos cm^{-1}		
Undisturbed	1.83	4.37	0.95	0.60	1.68	3.4	0.1	247	3.14	0.54	0.56	7.83	1.12
Disturbed													
overburden	88.75	16.95	3.76	1.33	6.00	4.4	10.1	1120	15.10	4.35	9.72	7.13	27.58
stockpiled ts	16.39	8.95	2.85	0.82	4.95	2.4	0.1	305	4.06	0.64	2.64	7.72	6.75
Reclaimed													
nontopsoiled													
1974	55.42	25.90	13.21	0.50	0.10	6.2	113.0	1409	17.56	5.67	8.66	6.79	12.53
1975	20.65	35.04	7.77	0.89	0.16	6.2	2.2	1459	13.96	5.69	4.63	6.67	4.36
1976	56.83	22.97	6.43	1.39	0.13	4.2	42.3	1368	16.53	5.06	6.52	6.85	14.82
topsoiled													
1978	3.21	4.92	1.17	0.33	0.39	1.6	3.4	404	4.49	0.44	0.83	7.73	1.84
1979	9.93	24.77	6.13	1.01	0.22	3.6	0.1	379	4.30	0.84	2.93	7.26	2.53
1980	7.70	21.30	7.89	1.50	0.43	4.5	2.8	453	5.21	0.87	2.75	7.12	2.02
1981	20.03	21.23	6.82	0.42	0.18	2.5	1.7	313	5.08	0.87	3.55	7.60	5.35
1982	39.01	21.67	4.23	0.66	0.33	2.2	25.4	709	10.88	1.17	6.92	7.63	10.84

[1]Water-soluble cations, EC, and pH determined from saturated paste extracts.
[2]P, NH_4-N, and NO_3-N determined from 1:5 water extract; Total Kjeldahl Nitrogen (TKN) determined by semi-micro-Kjeldahl.
[3]Organic matter determined by the Walkley-Black method.

Table 2. Fungal propagules and similarity coefficients occurring on an undisturbed soil, and disturbed and reclaimed coal mine spoils and soils.

Sites	Fungal propagules $10^3\ g^{-1}$	Similarity coefficients	
		SPCC[2]	SQCC
Undisturbed	6.7 d[1]		
Disturbed			
overburden spoil	3.4 d	26	28
stockpiled topsoil	11.1 d	47	38
Reclaimed			
nontopsoiled			
1974	52.0 abc	38	27
1975	44.0 bc	48	34
1976	67.0 ab	39	28
topsoiled			
1978	6.2 d	65	40
1979	35.0 bc	63	36
1980	58.0 abc	61	30
1981	81.0 a	67	38
1982	31.0 c	60	32

[1]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]SPCC = Sorensen's Presence Community Coefficient; SQCC = Sorensen's Quantitative Community Coefficient.

Table 3. Distribution and relative density of fungal genera of an undisturbed soil and of disturbed and reclaimed coal mine spoils and soils.[1]

Fungal groups	Undis-turbed	Disturbed		Reclaimed							
		Overbur-den	Stockpiled topsoil	Nontopsoiled			Topsoiled				
				1974	1975	1976	1978	1979	1980	1981	1982
Acremonium	1	0	0	0	0	0	0	14	2	0	0
Alternaria	1	2	0	0	0	1	0	0	1	6	8
Annellophorella	1	0	0	0	0	0	0	0	0	0	0
Aspergillus	5	1	13	26	68	83	37	14	30	16	10
Chaetomium	2	0	10	1	4	0	6	18	39	1	1
Chrysosporium	0	9	0	0	2	0	0	1	1	0	0
Cladosporium	0	0	1	1	0	0	0	0	0	0	0
Curvularia	15	0	3	0	5	4	3	2	13	6	2
Dreshlera	1	0	1	0	0	0	0	0	0	0	0
Fusarium	7	0	10	2	8	11	4	15	21	26	13
Humicola	5	0	0	0	2	0	5	0	0	0	0
Microascus	0	0	2	0	0	0	0	0	0	0	0
Myrothecium	1	0	0	0	0	0	1	0	38	17	14
Mortierella	1	0	0	0	0	0	0	3	0	0	0
Mucor	0	0	0	0	1	2	0	2	0	2	1
Mycelia sterilia	0	0	1	3	13	1	1	0	1	0	0
Penicillium	15	14	44	51	25	38	7	7	5	39	69
Phoma	5	0	0	0	0	0	1	0	0	0	0
Rhizopus	5	0	0	0	1	0	1	2	0	5	2
Sepedomium	0	0	1	0	0	0	0	1	1	0	0
Stachybotrys	3	0	1	1	0	0	4	8	11	25	18
Stemphyllium	0	0	0	0	0	0	1	0	4	0	0
Thielavia	1	0	0	0	0	0	0	0	0	0	0
Trichoderma	3	0	0	0	0	0	0	0	0	0	0
Trichurus	0	0	0	0	0	0	0	0	0	0	0
Unidentified											
Isolate No. 1	15	0	0	0	0	0	0	0	0	1	0
Isolate No. 2	3	0	0	0	0	0	0	2	1	0	0
No. of isolates	90	26	87	85	129	140	71	89	168	144	139
No. of genera	19	4	11	7	10	7	12	13	14	11	11
Fungal diversity	1.09	0.44	0.69	0.45	0.65	0.48	0.75	0.95	0.88	0.86	0.72
Evenness	0.85	0.74	0.66	0.53	0.65	0.56	0.69	0.85	0.76	0.82	0.69

[1]Relative density of isolates from six 1:1000 soil dilution plates.

higher plants. In this study, the important fungal genera with respect to the number of propagules isolated were Aspergillus, Chaetomium, Curvularia, Fusarium, Myrothecium, Penicillium, and Stachybotrys. Organisms like Penicillium, Fusarium, Myrothecium, Alternaria, and Stachybotrys decreased in densities with age, whereas organisms like Aspergillus increased with age. Acremonium, Chaetomium, and Curvularia were more numerous in the transitional areas than in the pioneer or in the more older topsoiled sites. Although an overlapping of fungal groups occurred among the topsoiled areas, each site had a different fungal dominant, thus indicating that a given fungal group had a closer relationship to a specific plant successional stage than to others. For example, Penicillium dominated the two youngest topsoiled sites (1982 and 1981), while Chaetomium dominated the next two oldest topsoiled sites (1980 and 1979), and Aspergillus was the dominant genus in the oldest topsoiled site (1978).

A different population size, diversity and fungal composition occurred in the nontopsoiled spoil areas in comparison with the topsoiled areas or to the undisturbed soil. In general, the topsoiled sites, and particularly the oldest topsoiled site (1978), had a fungal population size, diversity, and community composition more similar to the undisturbed soil than did any of the older nontopsoiled areas. The nontopsoiled areas had significantly higher populations of fungi but lower fungal diversities than the undisturbed soil (Tables 2 and 3). These results suggest that the nontopsoiled areas may not yet be stable, in that these areas may still have adverse soil chemical and physical properties (i.e., high salts and high clay contents). Aspergillus and Penicillium comprised 82% of the total isolates from these nontopsoiled areas. In contrast to the topsoiled areas where Penicillium was the dominant in the pioneer stage and Aspergillus was the dominant in the oldest topsoiled site (1978), the youngest nontopsoiled site (1976) was dominated by Aspergillus, whereas the oldest nontopsoiled site (1974) was dominated by Penicillium. In other words, the density of Aspergillus decreased with age, whereas Penicillium increased with age in the nontopsoiled areas.

The results of the present study and other studies (Tresner et al., 1954; England and Rice, 1957; Orpurt and Curtis, 1957; Mallik and Rice, 1966) indicated that the composition of fungal groups differed appreciably from one ecological area to the other, being influenced by plant cover and especially by soil factors. This investigation has demonstrated that each of the reclaimed nontopsoiled and topsoiled sites was different in terms of the number of fungal propagules, the diversity of fungal genera, and in the fungal genera present. The nontopsoiled areas contained a relatively low number of fungal taxa, while the topsoiled sites had a larger number of fungal genera. Aspergillus, Fusarium and Penicillium were common to both the nontopsoiled and topsoiled areas (Table 3). However, topsoiling and revegetation resulted in an increase in the occurrence of Stachybotrys compared to the nontopsoiled spoil areas, where this organism was found only once. The increased density of Stachybotrys may have been the result of the higher pH and the lower clay contents in the topsoiled sites when compared to the nontopsoiled sites. Mycelia sterilia, on the other hand, occured in higher densities in the nontopsoiled areas compared to the topsoiled sites. These differences between the two adjacent reclaimed areas would seem to add considerable weight to the concept of distinctive microfungal floras depending on the soil environment.

CONCLUSIONS

Fungal populations, diversity, and community composition were related to soil properties and site age. Higher levels of fungal propagules but low fungal diversity were characteristic of the nontopsoiled areas, whereas low fungal propagule levels but higher fungal diversity were characteristic of the topsoiled areas. The similarity between the undisturbed soil and the oldest topsoiled site (1978), in terms of the population of soil fungi, the relatively high diversity of fungal genera, and the similarity in the type and quantity of fungi isolated suggested that the fungal community may have begun to stabilize 4 years after topsoiling and revegetation. Many of the fungal genera isolated were common to all topsoiled areas. However, some appeared in considerably higher densities than others, suggesting that particular fungi had a closer relationship to a specific stage of vegetation. This may have been related to the changing and generally decreasing nutrient and organic matter contents in the topsoiled areas with the advance of plant establishment.

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Links to the papers are on the last page by first author.

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