SOIL FUNGAL COMMUNITY-PLANT RHIZOSPHERE INTERACTIONS DURING THE EARLY STAGES OF ECOSYSTEM DEVELOPMENT ON RECLAIMED COAL MINE SOILS[1]

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Abstract.--The reestablishment of microbial populations in the rhizosphere of plants growing on reclaimed coal mine soils is imperative to help accelerate revegetation by enhancing biological activity and nutrient cycling processes. In the present study, the microfungal community was evaluated in the rhizosphere and nonrhizosphere zones of galleta (Hilaria jamesii), alkali sacaton (Sporobolus airoides), and fourwing saltbush (Atriplex canescens) growing on a reclaimed coal mine soil after 0.25, 1.25, and 2.25 years of plant growth. Of the three plant species, the rhizosphere of fourwing saltbush had significantly higher fungal populations than the rhizosphere of galleta or alkali sacston 2.25 years after plant establishment. Although, the diversity of fungal groups in the rhizosphere and nonrhizosphere zones of all three plant species generally increased with plant age, the diversity of fungal groups in the rhizosphere of all three plant species were still lower than in the nonrhizosphere soil. Higher fungal populations and lower fungal diversities were generally related to higher nutrient contents in the rhizosphere, whereas lower fungal populations and higher fungal diversities were generally related to lower nutrient contents in the nonrhizosphere soil. Fungal groups changed between rhizosphere and nonrhizosphere zones, and among and within plant species as the age of the plants increased. Chrysosporium spp. dominated the rhizosphere and nonrhizosphere of all three plant species after 0.25 years. After 2.25 years, Aspergillus spp. dominated the rhizosphere of both grass species, whereas Penicillium spp. dominated the rhizosphere of fourwing saltbush.

INTRODUCTION

Research that has involved the belowground ecosystem in reclaimed coal mine soils has shown that changes in microbial populations and in the types of microorganisms involved may affect patterns of nutrient cycling activities during ecosystem development in soils which were not specifically from the rhizosphere (Fresquez et al. 1986, Parker et al. 1986). The potential interactions between plant roots and their associated microflora have been found to be important in other studies. Reviews by Rovira and Davey (1974) and Whipps and Lynch (1986) have indicated that different plant species excrete different types and amounts of organic materials (exudates) that influences the microflora composition in the rhizosphere. In turn, the rhizosphere microbial community provides

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substantial amounts of nutrients for plant use and acts as a buffer allowing plants to cope with environmental stress. Also, the benefits of an active rhizosphere environment are not only due to an increase of nutrients to the plant, but to the excretion of growth regulators such as gibberellins, auxins, cytokinins and indolyl acedic acid (Russell 1973).

The microbial community is often responsible for the modification of adverse soil properties (Alexander 1977). The reestablishment of microorganisms in the rhizosphere may be of particular importance in the revegetation and stabilization of mining spoils where the soil properties are unfavorable in the surrounding soil (away from the root) for microbial growth (Cundell 1977). The objective of this study was to determine the microfungal component and soil chemical properties in the rhizosphere and nonrhizosphere of native grass and shrub species growing in a reclaimed coal mine soil during the initial stages of plant community development.

MATERIALS AND METHODS

Rhizosphere and nonrhizosphere soil samples were collected from plots on which galleta (Hilaria jamesii), alkali sacaton (Sporobulus airoides) and fourwing saltbush (Atriplex canescens) were growing on a reclaimed coal mine soil 0.25, 1.25 and 2.25 years after initial plant establishment in July of 1982, 1983 and 1984, respectively. The disturbed area, reclaimed in 1982, was on the San Juan Coal Mine located 24 km west of Farmington, NM. This site was revegetated by grading and leveling the overburden spoil material, spreading stockpiled topsoil material 20 to 30 cm deep over the recontoured spoil, mulching with 4.5 metric tons of grass hay / ha crimped 15 cm deep, fertilizing with 72 kg N and 90 kg P/ ha, seeding with ten native plant species, and irrigating for two growing seasons (Fresquez et al. 1986).

At each sampling period, rhizosphere and nonrhizosphere samples for each plant species were collected randomly along each of four permanently located transects. Each replication of a plant species (4 replications per plant species) included sampling the rhizosphere (0 to 7 cm from the the stem of the plant), and nonrhizosphere (30 cm away from the stem of the plant). The rhizosphere was collected by carefully removing the plant(s), and placing the roots and clinging soil in a sterile plastic bag. The topgrowth was separated and placed in a separate bag, each time by removing the shootgrowth 6 mm above the crown of the plant. The nonrhizosphere soil samples were collected using a soil bucket auger (5 cm in diameter) to a depth of 13 cm. Both plant and soil samples were immediately cooled in an ice-chest for transport to the laboratory. At the laboratory, the rhizosphere soil samples were separated into soil and root material. All soil samples were passed through a 2-mm sieve. An aliquot from each sample was taken for chemical analysis at the New Mexico State University Soil and Water Testing Laboratory. All methods of analyses have been described previously (Fresquez and Lindemann 1983). Shootgrowth and rootgrowth were dried for 78 h at 60°C and weighed; root to shoot ratios (R:S) were determined from this data.

Rhizosphere fungal populations were estimated by methods similar to those described by Lochhead (1940) and Starkey (1958). Approximately 9 g of soil and the respective root system were placed in a preweighed 125-mL bottle containing 95-mL of sterile saline (0.85%) solution. For nonrhizosphere soil 10 g of soil was used. Dilutions were plated in triplicate on rose-bengal-streptomycin agar (Martin 1950), and incubated at 25°C in the dark for 7 days.

After diluting and plating, the roots were wsshed and removed. The wash water was collected in the original bottle and evaporated to dryness at 110°C. Both rhizosphere and nonrhizosphere soil samples were treated in this manner. The contents of the dilution bottles were weighed and the number of fungal propagules computed on a per gram of oven-dry material basis.

Variations in soil chemical properties and in the populations of fungi were analyzed using standard analysis of variance. Fungi were analyzed using natural log-transformed data. The Least Significant Difference (L.S.D.) test was used to compare soil chemical snd fungal populations means at the 5% probability level.

Fungal groups were isolated by evenly distributing 1 mL of a 10^{-3} dilution from a 10-g oven-dry weight equivalent sample in a Petri dish, adding cooled rose-bengal-streptomycin agar, and swirling for an even distribution. Ten plates were inoculated for each composited (4 replications per composite) soil sample, and incubated at 24°C in the dark for 7 days. After incubation, a portion of every colony appearing on the rose-bengal-streptomycin plates was transferred to a carrot agar medium by removing a portion of the agar containing hyphal tips. The colony was subcultured on a carrot agar plate to allow for maximum fruiting potential and identification. After a 4-day incubation period the colonies were identified using the taxonomic guides of Barnett and Hunter (1972), Barron (1968), Gilman (1968) and Domsch et al. (1980).

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

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

where p_i = the proportion of group i in the sample, and k = the number of groups. 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 fungal composition among the sample populations was calculated with Sorensen's presence community coefficient (SPCC), which was described by Mueller-Dombois and Ellenberg (1974) as:

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

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

RESULTS AND DISCUSSION

Galleta (Hija) and alkali sacaton (Spai) had significantly higher R:S ratios (0.27 and 0.36) than to fourwing saltbush (Atca) (0.03) after only 0.25 years of plant growth. After 2.25 years of plant growth, however, fourwing saltbush had a significantly higher R:S ratio (0.15) compared to galleta (0.06) or alkali sacaton (0.06).

In general, most soil properties in the rhizosphere, and especially in the nonrhizosphere soil of galleta, alkali sacaton and fourwing saltbush significantly decreased with reclamation age (table 1). This decline probably reflects the depletion of the added fertilizer and hay amendments, decreased plant productivity after irrigation was terminated and a reduced nutrient cycle as succession advances.

With respect to the rhizosphere and nonrhizosphere zones after only 0.25 years of plant growth, soil NO_3-N, organic matter (OM), electrical conductivity (EC), and the sodium absorption ratio (SAR) were significantly lower in the rhizosphere of galleta and alkali sacaton compared to the nonrhizosphere soil. The rhizosphere of fourwing saltbush had significantly lower P and higher SAR compared to the nonrhizosphere. It was not until after 2.25 years of plant growth that many soil chemical properties were significantly higher in the rhizosphere of galleta, alkali sacaton and fourwing saltbush compared to the nonrhizosphere soil. For example, the rhizosphere of galleta had significantly higher P, NH_4-N, TKN, OM and EC than in the nonrhizosphere soil. Similarily, the rhizosphere of alkali sacaton had significantly higher P, NO_3-N, OM, EC and SAR than in the nonrhizosphere soil, whereas, EC and SAR in the rhizosphere of fourwing saltbush were significantly higher than

Table 1.--Selected chemical properties in the rhizosphere (R) and nonrhizosphere (NR) of galleta (Hija), alkali sacaton (Spai) and fourwing saltbush (Atca) growing on a reclaimed coal mine soil varying in age.

Plant species	Phosphorus and nitrogen				Organic matter	EC	pH	SAR
	P	NH_4-N	NO_3-N	TKN				
	ug g^{-1}				g kg^{-1}	dSm^{-1}		
0.25-year-old								
Hija								
R	4.4 b[1]	1.0 b	4.5 b	464 b	9.8 d	3.7 b	7.9 a	10.6 b
NR	4.6 b	1.0 b	18.1 a	484 ab	11.4 c	6.8 a	7.6 c	14.9 a
Spai								
R	4.0 b	1.0 b	4.2 b	450 b	9.7 d	3.6 b	7.8 bc	10.2 b
NR	4.3 b	1.0 b	15.7 a	501 a	13.0 ah	6.7 a	7.8 bc	17.0 a
Atca								
R	3.5 c	1.0 b	5.2 b	430 bc	11.1 c	6.7 a	7.7 c	16.3 a
NR	4.1 b	1.2 ab	9.4 h	484 ab	10.1 d	6.4 a	7.8 bc	8.8 c
1.25-year-old								
Hija								
R	6.7	1.0	0.8	825	7.2	1.8	7.9	10.0
NR	3.5	0.5	1.3	335	7.8	5.0	7.8	11.3
Spai								
R	2.4	0.2	1.8	389	11.1	1.8	8.0	9.1
NR	1.8	0.9	1.3	361	9.5	3.4	7.8	8.0
Atca								
R	1.8	0.9	0.9	355	7.5	1.7	8.2	16.2
NR	4.1	1.2	1.4	402	10.8	3.7	8.0	14.5
2.25-year-old								
Hija								
R	4.5 b	1.4 a	1.1 c	502 a	14.1 a	1.6 c	7.9 ab	6.7 d
NR	3.5 c	0.7 bc	1.0 cd	362 c	12.1 bc	0.8 d	7.9 ab	6.4 d
Spai								
R	7.3 a	0.7 bc	1.3 c	402 c	10.8 cd	1.5 c	8.0 a	8.1 c
NR	2.9 cd	0.9 c	0.8 d	362 c	8.5 e	0.8 d	8.0 a	6.0 d
Atca								
R	2.4 d	0.7 bc	0.8 d	362 c	9.2 de	2.9 b	7.9 ab	10.6 b
NR	3.5 c	1.6 a	1.0 cd	428 bc	8.5 e	1.1 cd	8.0 a	9.5 c

[1]Means within the same column followed by the same letter are not signifiantly different at the 0.05 level by the LSD test.

in the nonrhizosphere. Organic matter was the only other soil parameter that was generally higher in the rhizosphere of fourwing saltbush than in the nonrhizosphere, but the values were not significantly different, however. Soil P and especially OM in the rhizosphere of both galleta and alkali sacaton were significantly higher over those soil properties in the nonrhizosphere. The accumulation of many soil chemical properties, particularly P, are found to be higher in the rhizosphere in the presence of microorganisms compared to the nonrhizosphere (Bowen and Rivera 1966).

Populations of fungi in the rhizosphere and nonrhizosphere zones of galleta and alkali sacaton, and in the nonrhizosphere soil of fourwing saltbush generally decrease with reclamation age (table 2). Of the three plant species, the rhizosphere of fourwing saltbush had significantly higher fungal populations than the rhizosphere of galleta and alkali sacaton. Also, the significantly higher fungal populations and lower diversity of fungal groups in the rhizosphere of fourwing saltbush compared to the nonrhizosphere soil after 2.25 years of plant growth suggests a root environment highly conducive to fungal growth.

A significantly higher fungal population in the rhizosphere of fourwing saltbush compared to the nonrhizosphere cannot be attributed to the measured soil chemical properties alone, as EC and SAR were the only soil chemical properties that were significantly higher in the rhizosphere

Table 2.--Fungal propagules ($10^{-3}g^{-1}$) in the rhizosphere (R) and nonrhizosphere (NR) of galleta (Hija), alkali sacaton (Spai) and fourwing saltbush (Atca) growing on a reclaimed coal mine soil varying in age.

	Hija	Spai	Atca
0.25-year-old			
R	414 a A[1]	460 b A	360 c A
NR	110 b	91 c	82 d
1.25-year-old			
R	148 b C	879 a B	1929 b A
NR	42 c	24 d	27 d
2.25-year-old			
R	71 c B	91 c B	14339 a A
NR	13 d	10 d	28 d

[1]Means within the same column followed by the same lower case letter are not significantly different at the 5% probability level by the L.S.D. test. Similarly, means within the same horizontal row followed by the same upper class letter are not significantly different.

Table 3.--Fungal diversity (H) and evenness (J) in the rhizosphere (R) and nonrhizosphere (NR) of galleta (Hija), alkali sacaton (Spai) and fourwing saltbush (Atca) growing on a reclaimed coal mine soil varying in age.[1]

	Hija		Spai		Atca	
	H	J	H	J	H	J
0.25-year-old						
R	0.68	0.57	0.72	0.60	0.82	0.65
NR	0.79	0.69	0.75	0.61	0.93	0.74
1.24-year-old						
R	0.93	0.81	0.73	0.61	0.40	0.37
NR	0.78	0.62	0.68	0.63	1.01	0.79
2.25-year-old						
R	1.04	0.76	0.78	0.70	0.98	0.73
NR	1.14	0.85	1.15	0.82	1.15	0.83

[1]Diversity calculated from isolatea occurring on ten 1:1000 soil dilution plates.

of fourwing saltbush compared to the nonrhizosphere. Also, the amount of OM in the rhizosphere of fourwing saltbush after 2.25 years of plant growth was generally lower than the OM in the rhizosphere of galleta or alkali sacaton. A comparison of R:S ratios, on theother hand, shows that fourwing saltbush after 2.25 years of plant growth had an R:S ratio 2.5 times higher than either galleta or alkali sacaton. The amount of C material released by plant roots may depend upon root weight (Wood 1987). However, Bamberg et al. (1973) have shown that the amount of fixed C translocated to the roots by shrub species, including fourwing saltbush was considerably lower than grass species. Thus, the stimulation of the fungal community in the rhizosphere of galleta or alkali sacaton may have been partially due to the amount of root biomass, which may have influenced the type rather than the amount of C released as exudates by fourwing saltbush.

The diversity and evenness of fungal groups in the rhizosphere and nonrhizosphere of galleta, alkali sacaton and fourwing saltbush generally increased with reclamation age (table 3). After 2.25 years of plant growth, however, the diversity of fungal groups in the rhizosphere of galleta, alkali sacaton and fourwing saltbush were generally lower compared to the diversity in the nonrhizosphere soil. Generally, higher fungal populations and lower fungal diversities are related to either adverse soil or surface conditions, or to soils high in nutrient levels (Guillemat and Montegut 1960, Joffe 1967, Dennis and Fresquez, unpublished data). In contrast, lower fungal populations and higher fungal diversities are associated with moderately low nutrient levels (Dennis and Fresquez, unpublished data). Thus, while the soil nutrient status was decreasing with reclamation age, the rhizosphere of galleta, alkali sacaton and fourwing saltbush provided a more conducive environment to the fungal community than in the nonrhizosphere where there were less available nutrients.

The overall composition of fungal groups changed between the rhizosphere and nonrhizosphere zones, and among and within plant species as the age of the plants increased in age. With respect to the composition of fungal groups between the rhizosphere and nonrhizosphere zones of galleta, alkali sacaton and fourwing saltbush, it was found that the percent similarity between these zones decreased as the age of galleta and alkali sacaton increase. In contrast, the similarity in the composition of fungal groups between the rhizosphere and nonrhizosphere of fourwing saltbush increased slightly from 72 to 78% with plant age. This suggested that the rhizosphere of fourwing saltbush may have influenced the types of fungal groups in the nonrhizosphere soil 2.25 years after plant establishment more than the rhizosphere of galleta or alkali sacaton. The rhizosphere of galleta, alkali sacaton and fourwing saltbush changed among plant species as the plants increased in age from 0.25 to 2.25 years. For example, the percent similarity (SPCC) between plant species after 0.25 years of plant growth was high ranging from 71 to 88%. After 2.25 years of plant growth, the percent similarity of fungal groups in the rhizosphere between plant species decreased, ranging from 61 to 67%. Also, an overall decrease in the percent similarity of fungal groups isolated in the rhizosphere within the same plant species occurred with age. As the plants increased in age from 0.25 to 2.25 years, the fungal composition in the rhizosphere of galleta decreased to 56%; alkali sacaton decreased to 55%; and fourwing saltbush decreased to 60%.

Different fungal groups dominated the rhizosphere and nonrhizosphere of galleta, alkali sacaton and fourwing saltbush as the plants increased from 0.25 to 2.25 in age (table 4). Chrysosporium spp. dominated the rhizosphere and nonrhizosphere of galleta, alkali sacaton and fourwing saltbush 0.25 years after plant establishment. After 2.25 years of plant growth, Aspergillus spp. dominated the rhizosphere of galleta and alkali sacaton, while Penicillium spp. dominated the rhizosphere of fourwing saltbush. Penicillium spp. accounted for 33% of the total isolates from the rhizosphere of fourwing saltbush and was probably the fungal group most responsible for the majority of the enumerated propagules in the rhizosphere of fourwing saltbush 2.25 years after plant establishment (table 4).

Table 4.--Major fungal groups in the rhizosphere (R) and nonrhizosphere (NR) of galleta (Hija), alkali sacaton (Spai) and fourwing saltbush (Atca) growing on a reclaimed coal mine soil varying in age. (% of total isolates).

	Hija	Spai	Atca
0.25-year-old			
R	Chrysosporium (43)	Chrysosporium (44)	Chrysosporium (40)
NR	Chrysosporium (44)	Chrysosporium (46)	Chrysosporium (33)
1.25-year-old			
R	Chaetomium (28)	Penicillium (60)	Myrothecium (77)
NR	Chaetomium (50)	Penicillium (25)	Chaetomium (14)
2.25-year-old			
R	Aspergillus (31)	Aspergillus (65)	Penicillium (33)
NR	Aspergillus (25)	Chaetomium (28)	Aspergillus (24)

CONCLUSIONS

These data show that an active rhizosphere environment may be a vital attribute to the overall success of revegetation on reclamined coal mine soils. Each plant species, however, may affect or contribute differently to the overall success of revegetation. Of the three plant species, the rhizosphere of the grass species had significantly higher N and P, and OM accumulation than the rhizosphere of fourwing saltbush. On the other hand, the rhizosphere of fourwing saltbush had significantly higher EC and SAR than the rhizosphere of the grass species 2.25 years after plant establishment. The rhizosphere of fourwing saltbush, however, was more conducive to fungal growth than galleta or alkali sacaton. As a result of this, the rhizosphere of fourwing saltbush may contribute to greater decomposition and soil aggregation processes more deeply into the soil profile than the rhizosphere of the two grass species. In contrast, the rhizosphere of galleta and alkali sacaton may contribute more to nutrient cycling processes, particularly N and P, and OM accumulation more near the soil surface than the rhizosphere region of fourwing saltbush.

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