PHYSIOLOGICAL DIVERSITY OF RANGELAND SOIL MICROBIAL COMMUNITIES AS AFFECTED BY RETORTED OIL SHALE[1]

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Abstract.--Relative changes in rhizosphere, rhizoplane, and non-rhizosphere microbial physiological diversity in response to retorted shale energy residuals were evaluated in a greenhouse experiment. Agropyron smithii Rydb. and Atriplex canescens (Pursch) Nutt. were grown in a control soil, retorted shale or soil over shale treatment.

Bacteria obtained from retorted shale exhibited a wider range of tolerance to alkalinity and salinity, along with decreased growth on amino acid substrates, as compared with those bacteria from soil and soil over shale. Root-associated bacterial counts were higher in spent shale, as compared to the soil control. Retorted shale had no observable effect on the number of non-rhizosphere bacteria. The calculated physiological diversity of bacteria was only slightly affected by retorted shale. However, results of bacterial community similarity measurements suggested that non-rhizo- sphere bacteria were impacted more by retorted oil shale than root-associated bacteria.

INTRODUCTION

Plants and their associated soil microbial communities respond to environmental stress in many subtle and interactive ways. Successful revegetation on surface-disposed retorted oil shale or on topsoil placed directly over retorted shale may be dependent on the development of a functional microbial belowground community. The importance of a functional microbial community in a plant-soil system has been well documented (Alexander 1977; Baver et al. 1972). Microorganisms promote soil formation (Buol et al. 1980) and microbial processes contribute to the accretion of soil organic matter and the modification of adverse soil properties (Cundell 1977). It is apparent that reestablishment of a functional belowground component will be necessary to ensure successful long-term restoration of disturbed ecosystems.

Field studies have shown a decrease in microbial activity in surface soils that directly overlay Paraho processed oil shale (Sorenson et al. 1981). In a laboratory study, Hersman and Klein (1979) showed that microbial activity was decreased in retorted shale-soil mixtures, as compared with normal soils. Rogers et al. (1982) investigated the initial microbial colonization of retorted shale in a field lysimeter study. They found that, two months after disposal, surface horizons of retorted shale were dominated by a single Micrococcus species.

Little information is available on mechanisms responsible for decreased microbial activity and diversity often associated with soils that overlay retorted shale or in retorted shale-soil mixtures. Retorted shale, either directly or indirectly, may have caused a selective reduction in microbial numbers or could have effected a functional change in an unchanged microbial population.

A greenhouse pot experiment was initiated to evaluate the effects of retorted shale on the development of rhizosphere, rhizoplane, and non-rhizosphere microorganisms. Selected reclamation plant species that have shown some potential for establishment on these materials were utilized in this study.

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MATERIALS AND METHODS

Greenhouse Study

Surface soil material (0-15 cm depth) was obtained from the off-site control area of the Intensive Study Site (ISS) located in the Piceance Basin in northwestern Colorado (Redente and Ruzzo 1979). The Intensive Study Site is maintained by the Range Science Department at Colorado State University. Native soils of this region have been mapped as a soil complex that consisted of a Rentsac channery loam, Piceance fine sandy loam, and Yamac loam (USDA-SCS 1975). Soil was passed through a 6 mm mesh screen and was stored in plastic bags at $6^{o}C$ until ready for use in the greenhouse pot experiment.

Retorted oil shale was obtained from the Laramie Energy Technology Center, Laramie, Wyoming. Oil shale mined from Anvil Points, Colorado was retorted via a simulated vertical modified _in situ_ batch process. After retorting, the spent shale was passed through a hammer mill and crushed to a size ranging from fine to 10 cm. The retorted shale was leached with approximately three pore volumes of water in an attempt to reduce its initially high electrical conductivity.

Rosana western wheatgrass (_Agropyron smithii_), and fourwing saltbush (_Atriplex canescens_) were grown on the plant growth media that follows:

1. Soil control
2. Retorted oil shale
3. 8.5 cm soil over 8.5 cm retorted oil shale

In addition, each plant growth medium received a 2.5 cm surface layer of a vermiculite, perlite, and composted pine bark mixture, which served as a germination bed. A split-block with four blocks was set up to contain all possible combinations of plant species and plant growth medium treatments.

Soil, retorted oil shale, and surface layer germination materials were added to 20-cm deep plastic pots, which had been previously surface sterilized in a solution of Wescodyne. All seeds were surface sterilized for three minutes in a 10% Chlorox solution before they were planted. All treatments were fertilized with an equivalent of 110 kg nitrogen (N)/ha and 90 kg phosphorus (P)/ha. One-half of the N, as ammonium nitrate, was applied one week after emergence; the balance was applied one and a half months later. Phosphorus, as triple super phosphate, was mixed into the soil and shale materials prior to seeding. The western wheatgrass and fourwing saltbush stands were thinned to ten and four plants, respectively, per pot after emergence. Pots were maintained near field capacity under natural light in a greenhouse from June through August 1983. After a total growth period of three months, plants were harvested for microbial population analyses.

Microbial Analyses

Viable Enumeration

Viable enumerations from all three microbial population sources, i.e., rhizosphere, rhizoplane, and non-rhizosphere, were estimated by dilution and spread plate techniques (Wollum 1982) on material obtained from soil, retorted shale, and the soil layer from the soil over shale treatment. Dilutions of non-rhizosphere plant growth media were plated in triplicate on sodium caseinate agar for aerobic heterotrophic bateria (Society of American Bacteriologists 1957). Plates were incubated at room temperature in the dark and were counted after a two-week incubation.

Rhizosphere and rhizoplane microorganisms were obtained with techniques similar to those described by Louw and Webley (1959). One plant of each species was randomly selected from each of the three plant growth medium treatments. The surface germination layer was removed and discarded and the root systems were gently extracted from the soil or shale. The roots, along with the soil that adhered to them, were transferred to a pre-weighed 250 ml flask that contained 99 ml phosphate buffer and were shaken on a New Brunswick Scientific Model G2 shaker for five minutes at 200 rpm. The roots were aseptically removed with alcohol-flamed forceps from the flask that contained rhizosphere soil and were placed in a pre-weighed 50 ml dilution tube, which contained 5.0 g glass beads (3 mm dia.) and 10 ml phosphate buffer. The rhizoplane dilution tube was shaken in a horizontal position for 20 minutes on the above mentioned mechanical shaker (Sherwood and Klein 1981). Rhizosphere and rhizoplane micro- organisms were plated and counted as in the non- rhizosphere fraction. Dry weights of rhizosphere soil and rhizoplane roots were obtained by evaporating the initial dilution blanks overnight in a forced draft oven maintained at $105^{o}C$ (Wollum 1982).

Characterization of Bacterial Isolates

Aerobic heterotrophic bacteria were classified by a numerical taxonomic approach (Sneath 1957). A total of 450 well-isolated rhizosphere, rhizoplane, and non-rhizosphere bacterial colonies were randomly obtained from the original viable enumeration samples. Bacterial colonies were maintained on 18 replicate sodium caseinate agar plates, with 25 isolates per plate.

Bacterial isolates were characterized by the tests that follow:

1. Physiological tests: growth at pH 7 on sodium caseinate medium modified with dipotassium hydrogen phosphate (K_2HPO_4) buffer; growth at pH 10 on sodium caseinate medium modified with disodium hydrogen phosphate-sodium hydroxide (Na_2HPO_4-NaOH) buffer; growth on sodium caseinate, pH 7, plus the addition of 1.0%, 2.5%, 5.0%, 7.5%, or 10.0% w/v sodium chloride (NaCl); exoenzyme production in Petri dishes; amylase on 1.0% starch medium (Wollum 1982); proteolysis on

peptone/beef extract/gelatin medium (Wollum 1982); Tween 80 esterase on Sierra medium (Sierra 1957); gelatinase by the method of Frazier (1926); chitinase on a medium that contained sterile precipitated chitin (Skujins et al. 1965); cellulase on a 1.0% cellulose-azure modified Pettersson medium (Smith 1977), without additional carbon source.

2. Nutritional tests: growth on 0.1% glycine or methionine as sole carbon sources (Colwell and Weibe 1970).

Isolates were coded "1" for a positive and "0" for a negative response. Bacterial isolates were clustered with CLUSTAN (Wishart 1978), a cluster analysis computer package. A simple matching coefficient was utilized as the similarity coefficient. Clusters were formed by means of average linkage, which is equivalent to the unweighted pair-group (UPGMA) method of Sokal and Michener (1958). A cluster generated from CLUSTAN in this study was synonymous to a bacterial cluster, i.e., a group of n bacterial isolates functionally related to each other at some preselected level of similarity.

Bacterial Diversity Analysis

Bacterial diversity was calculated by the rarefaction method. Isolates were pooled from samples collected from western wheatgrass and fourwing saltbush. Simberloff (1978) developed a computer program that calculated the expected number of species or groups present in a subsample of individuals drawn from the total population. The raw data, i.e., total number of bacterial clusters and the number of isolates per cluster, needed to run the rarefaction program was obtained from the cluster analysis program. Bacterial isolates were clustered at an 85% level of similarity. This level was selected following procedures similar to those given by Mills and Wassel (1980). Comparisons were made among growth medium treatments, within each bacterial population source (i.e., rhizosphere, rhizoplane, and nonrhizosphere).

Bacterial Community Similarity

Community similarity was determined from the pooled bacterial sample with Sorensen's Index (Pielou 1977). Within each bacterial population source, pairwise comparisons were made among the plant growth media. The binary unit character data were reclustered for each comparison by the above-noted procedures. Values of the index range from zero (no common cluster) to one (all clusters in common).

Statistical Analyses

Data were analyzed with the Statistical Package for the Social Sciences (Nie et al. 1975) computer package. Analysis of variance programs (ANOVA) were performed on microbial enumeration data. Significant effects (i.e., F-statistic at $p < 0.05$) of the plant growth medium were further evaluated by the least significant difference method, with a 95 % confidence interval. Bacterial physiological data were evaluated for effects of plant growth medium on unit character occurrence by the Kruskal-Wallis one-way analysis of variance test.

RESULTS AND DISCUSSION

Characterization of Plant Growth Media

Results from selected chemical analyses of the plant growth media are given in table 1. The control soil was non-saline and non-sodic, with a near neutral pH. The predominant cations extracted from the soil solution were the basic cations of calcium (Ca) and magnesium (Mg), reflected in the relatively low SAR value. High alkalinity and sodicity characterized the leached retorted shale, with sodium (Na) and potassium (K) as the predominant cations in solution.

Table 1. Chemical characteristics of control soil and leached retorted oil shale prior to plant growth.

Measurement	Control soil	Leached shale
pH[1]	7.4	12.6
EC (mmhos/cm)[2]	0.4	15.4
SAR	0.38	66.8
Cations (meq/l)[2]		
Ca	6.8	0.9
Mg	1.0	<0.1
Na	0.6	47.0
K	0.1	13.2
Anions (meq/l)[2]		
HCO_3	5.6	0
CO_3	0	6.6
OH	0	54.9
Cl	0.3	1.1
SO_4	ND	5.0
Nitrate-N (ppm)[3]	8	2
P (ppm)[3]	2	19
K (ppm)[3]	45	442
Organic matter (%)	1.1	0.2

[1]Paste
[2]Saturation extract
[3]NH_4HCO_3-DPTA extract
ND Not determined

Microbial Enumeration Responses

Distinct differences existed in microbial population responses to retorted shale energy residuals. Rhizoplane and rhizosphere populations responded in a similar manner to spent shale, which was different from the pattern observed in non-rhizosphere populations. Rhizosphere and rhizoplane viable bacteria were significantly elevated by the presence of retorted shale, while these same groups of microorganisms were not affected by the plant growth medium in the non-rhizosphere (table 2). The different response patterns that were observed may be indirectly influenced by the presence of different plant species. Plant root exudates have been shown to support a numerous and diverse microbial community (Alexander 1977). The amount of materials released from roots generally increases with plant stress (Hale and Moore 1979). The highly sodic-saline nature of the retorted shale growth medium might expose plants to a certain degree of environmental stress which in turn could lead to higher levels of plant root exudation. Microorganisms that might use these substrates could be expected to proliferate.

Bacterial Diversity

Bacterial diversity increases slightly with spent shale present in the growth medium (table 2). If exudation was increased in roots subjected to retorted shale, more microbial substrates may have been available for utilization by the indigenous microflora. However, differences in diversity among plant growth media were not that great. Most bacterial isolates were distributed in a few major clusters (table 3). Several rarer bacterial clusters consisted of a few isolates, while several one-isolate cluster groups were common among growth medium treatments.

Although the total number of physiological groups of bacteria were not significantly affected by growth medium treatment, cluster profiles yielded distinct differences in bacterial groups that comprised the clusters (fig. 1). The tests that monitored growth on amino acid substates, tolerance to salinity and tolerance to alkalinity were clearly affected by the type of plant growth medium used. Amino acid utilization was lower, and tolerance to salinity and alkalinity was higher in isolates obtained from the retorted shale growth medium than from the soil control. Exoenzyme activity of selected hydrolases in bacteria from spent shale was variable. More proteolytic and lipolytic bacteria were associated with the retorted oil shale plant growth medium, while amylase, gelatinase, and chitinase activity was depressed in this same treatment, compared to the soil control. No cellulase activity was observed in any bacteria. However, exoenzyme production was not affected by the presence of retorted shale in the plant growth medium as much as the unit characters of alkalinity, salinity, and amino acid utilization were. Frankenberger and Bingham (1982) found, in a study where the influence of salinity on soil enzyme activity was evaluated, that a group of selected hydrolases

Table 2. Population densities and physiological diversity of bacteria in response to plant growth medium treatments.

Sample	Bacterial[1] plate count	Total no. of isolates	Total no. of clusters	Expected no. of clusters[2]
Rhizosphere				
Soil	8.2 b[3]	39	8	7.8 (.4)[4]
Shale	9.4 a	46	11	9.6 (.9)
Soil/Shale[5]	8.5 b	45	11	10.2 (.8)
Rhizoplane				
Soil	8.6 b	41	8	7.2 (.8)
Shale	9.6 a	48	9	8.3 (.7)
Soil/Shale	8.8 b	47	9	8.1 (.8)
Non-rhizosphere				
Soil	6.3 a	33	9	9.0 (0)
Shale	6.6 a	46	9	8.3 (.7)
Soil/Shale	6.6 a	38	13	12.3 (.7)

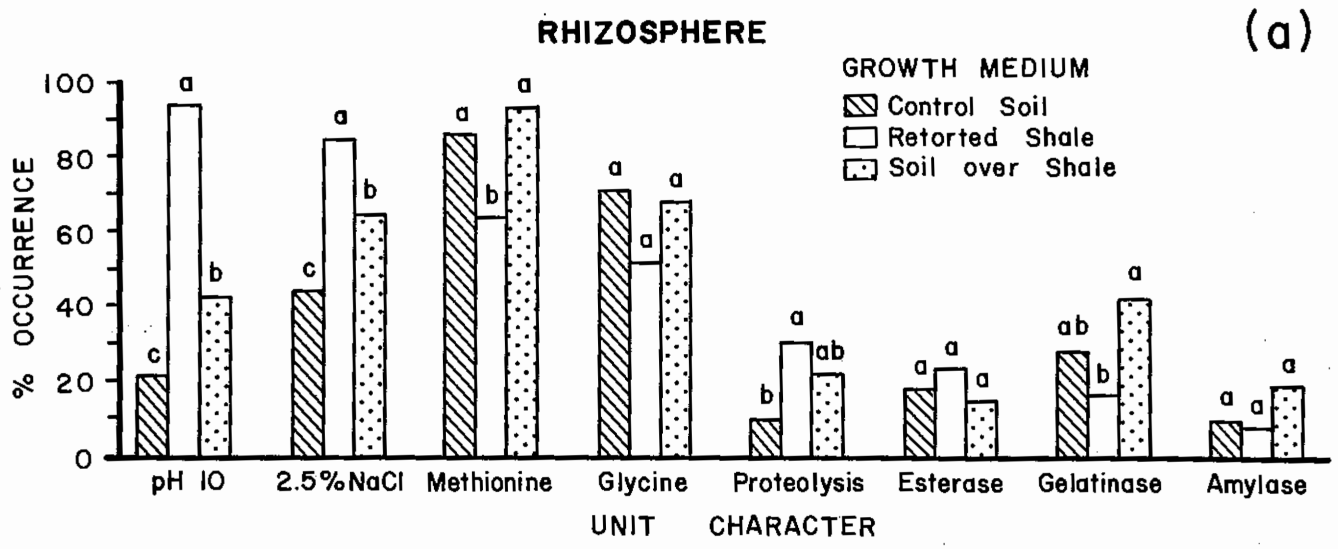
[1]Rhizosphere and non-rhizosphere: log colony-forming units/g dry soil. Rhizoplane: log colony-forming units/g dry root.

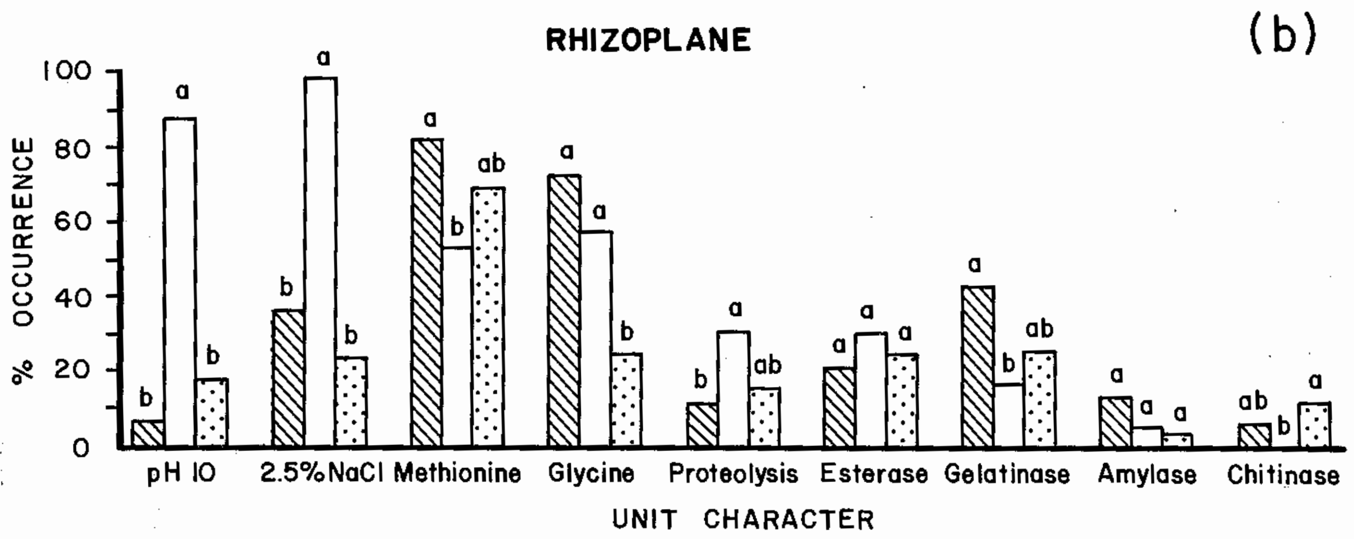
[2]Rarefied with 33 isolates.

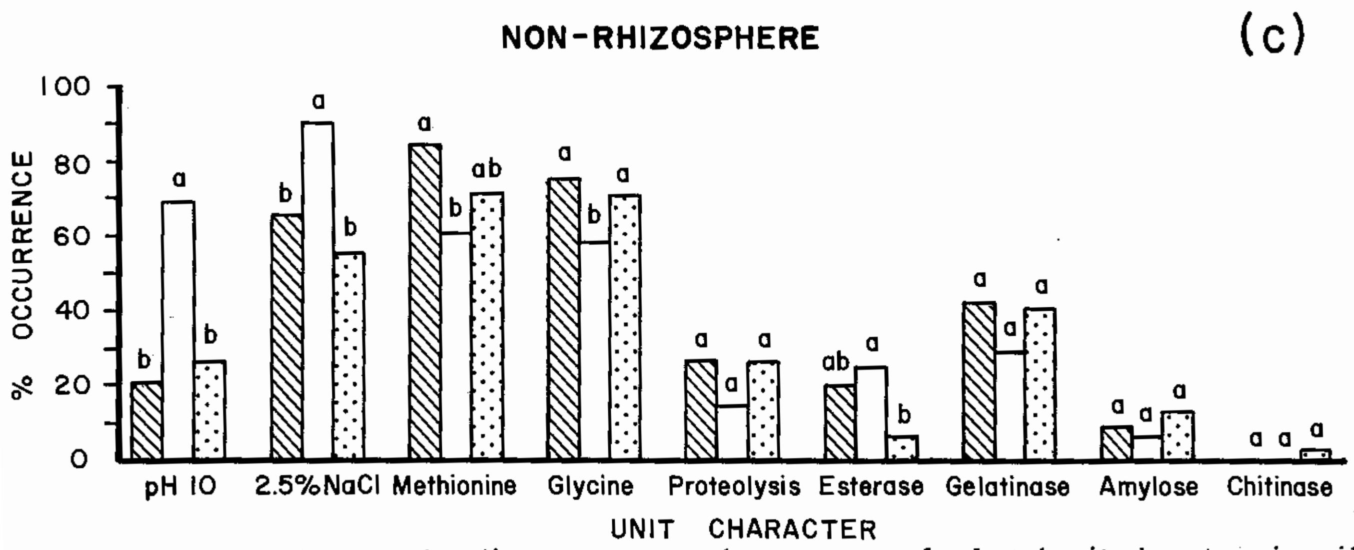
[3]Means with different letters within a bacterial population source are significantly different ($p < .05$).

[4]Standard deviation of the mean.

[5]Denotes soil over retorted oil shale treatment.







'igure 1. Effects of plant growth medium treatment on the occurrence of selected unit characters in soil bacterial isolates: a) rhizosphere, b) rhizoplane, c) non-rhizosphere. Different letters within a unit character are significantly different ($p < 0.05$).

Table 3. Distribution of isolates among the bacterial clusters.

Sample	Distribution of isolates among clusters[1]
Rhizosphere	
Soil	13, 9, 7, 3, 2(3), 1
Shale	16, 12, 5, 3, 2(3), 1(4)
Soil/Shale[2]	12, 10, 5, 4(2), 2(4), 1(2)
Rhizoplane	
Soil	19, 11, 5, 2, 1(4)
Shale	16, 12, 4(3), 3(2), 1(2)
Soil/Shale	28, 4(2), 3, 2(3), 1(2)
Non-rhizosphere	
Soil	9, 6, 5(2), 3, 2, 1(3)
Shale	15, 10, 7, 4(2), 2(2), 1(2)
Soil/Shale	9, 6(2), 3(2), 2(3), 1(5)5

[1]Numbers outside parentheses represent the number of isolates in the bacterial cluster. Numbers inside the parentheses represent the number of bacterial clusters that size and are shown for cluster frequencies greater than 1.

[2]Denotes soil over retorted oil shale treatment.

were less inhibited than the oxido-reductases that were screened.

It is reasonable to expect that, because of the high alkalinity and salinity of retorted shale, organisms able to survive in this material must also be tolerant of these two environmental conditions. However, spent shale isolates also maintained an ability to grow at pH 7 with no added salt. This suggested that excess salt and high pH were not required for growth of these isolates. Soil isolates appeared to be more specialized than spent shale populations and were restricted to a narrower range of pH and salt addition levels.

Bacterial Community Similarity

Measurements of community similarity were determined to evaluate the degree of impact retorted oil shale had on microbial community structure. Similar diversities did not necessarily imply that the microbial assemblages were composed of the same groups. Pairwise comparisons of microbial clusters yielded a consistent finding; bacterial clusters from retorted shale and the soil control were the most dissimilar, independent of population source (table 4). Within the growth medium treatments, rhizosphere, and non-rhizosphere populations shared a number of common clusters which were equal to or greater than the number of unique clusters. Wassel and Mills (1983) found distinct similiarities in freshwater and sediment bacterial communities that were exposed to varied concentrations of acid mine drainage. They suggested that this effect may have been caused by the selectiveness of the enumeration medium. The soil that overlay the spent shale in the soil over shale treatment was the same material as the control soil. It would be feasible to expect that these two treatments would share a common pool of organisms.

Table 4. Measurements of bacterial community similarity obtained from pairwise comparisons of plant growth media from each population source.

Population Source / Growth Media[1] A	B	No. of unique clusters (isolates) A	B	No. of common clusters	Sorensen's Index
Rhizosphere					
Soil	Soil/Shale[2]	5 (8)	6(13)	7	0.56
Shale	Soil	5(22)	4(11)	5	0.53
Soil/Shale	Soil	4 (8)	3 (9)	7	0.67
Rhizoplane					
Soil	Soil/Shale	6(19)	8(33)	3	0.30
Shale	Soil	8(32)	6(28)	2	0.22
Soil/Shale	Soil	4 (7)	3 (4)	7	0.67
Non-rhizosphere					
Soil	Soil/Shale	3(13)	5 (8)	7	0.64
Shale	Soil	4 (9)	4 (9)	6	0.60
Soil/Shale	Soil	6 (8)	2 (4)	6	0.60

[1]Plant growth media under column A had a greater proportion of retorted oil shale than those under column B for each pairwise comparison.

[2]Denotes soil over retorted oil shale treatment.

Community similarities measurements indicated two apparent responses to the presence of processed oil shale that were dependent on bacterial population source. Rhizosphere and rhizoplane populations from the soil over shale and soil only medium exhibited the highest level of relative similarity. Populations in the soil over shale treatment were more similar to those from spent shale among the non-rhizosphere comparisons. These results suggested that activity in non-rhizosphere bacterial populations may be more susceptible to retorted shale than populations from the rhizosphere or rhizoplane. Direct contact with spent shale may not be a necessary prerequisite to alter community functional status. The root zone may have provided a buffer against the retorted oil shale for root-associated microbial populations and may have modified the region proximal to the root.

CONCLUSIONS

The effects of retorted oil shale energy residuals on microbial population counts and bacterial physiological diversity were investigated. The benefit of topsoil addition to ameliorate these effects was also evaluated.

Specific conclusions that could be drawn from this study were:

1. Viable microbial responses to retorted shale were affected by population source. This implied that microbial population levels were influenced by interactions between plant roots and the retorted shale growth medium.

2. The presence of retorted shale in the plant growth medium had no detrimental effect on the total calculated bacterial physiolgoical diversity as determined by rarefaction.

3. Comparisons of bacterial community similarity varied with the population source. Results suggested that non-rhizosphere populations may be more sensitive to retorted oil shale, even in soils that overlay shale, than root-associated populations.

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