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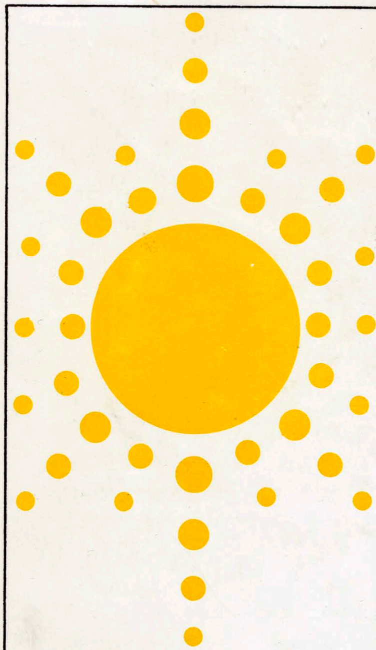
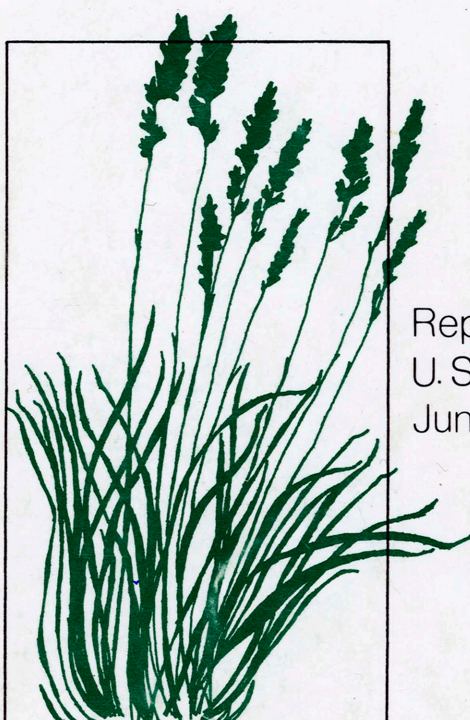


Revegetation Studies On Oil Shale Related Disturbances In Colorado

Research Report
By
Colorado State University



Report Submitted to
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June 1982

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Revegetation Studies On Oil Shale Related Disturbances In Colorado

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RESTORATION OF NATURAL FUNCTIONING ECOSYSTEMS ON DISTURBED LANDS

PROGRESS REPORT 1981-1982

ABSTRACT

An interdisciplinary research project was initiated in 1976 to provide both basic and applied information that would aid in the reestablishment of natural functioning ecosystems on land disturbances associated with energy development. The approach included field, laboratory, and greenhouse experiments designed to provide both structural and functional information about disturbed ecological systems in the semiarid west. This report presents results from the sixth year of the study.

The degree of soil disturbance substantially influences the rate of natural plant succession. Mixing soil horizons reduces the probability that perennial species would contribute significantly to rapid invasion and increases the probability that annual weeds would be the more prominant invader. In general, introduced and native seed mixtures produce similar amounts of aboveground biomass. However, introduced species were slightly more resistant to invasion by volunteer species. Fertilizer is effective in increasing seeded grass and shrub production, and the effect may still be evident five years following initial application. The use of 90 cm of topsoil and 60 cm of topsoil over a capillary barrier proved to be the best treatments for supporting plant species over Paraho retorted shale after four years. The manipulation of topsoil depth and the use of a capillary barrier may ultimately prove useful as a managment tool in modifying the ultimate plant community structure.

Soil disturbance may have a long-lasting effect on belowground processes; with better maintenance of the surface soil and decomposer subsystems such effects can be minimized. Monitoring of the nitrogen cycle has shown that a single fertilizer treatment at the beginning of reclamation of a disturbed soil can have long-lasting effects. The results of nitrification and ammonium volatilization trials also suggest that only a minor part of the added N will be lost due to volatilization. As reestablished plant communities mature, it appears that there is a major shift in biogeochemical cycling with the reallocation of nitrogen and phosphorus to less available pools and subsequent decreases in the cycling of these components.

Storing topsoil may have negative effects upon populations of viable mycorrhizal fungi. Topsoil storage for three years clearly indicates significant decreases in the mycorrhizal inoculum potential (MIP) of the topsoil when the soil is left unplanted. However, when the topsoil is planted with mycorrhizal plant species, the MIP values are maintained in the upper 90 cm of the storage pile. Functional mycorrhizae do not form in Paraho and Union decarbonized shales after several growing seasons, but there does not appear to be any adverse effect in terms of MIP of the soil that retorted shale exerts on soil placed over these shales. TOSCO II spent shale, when leached and amended with fertilizer, allowed mycorrhiza formation in the upper 30 cm of the growth media profile but inhibited mycorrhiza formation at deeper depths.

In the study of ecogenetic variability in native shrubs, it has been found that ecotypic differentiation is not strongly evidenced for the populations studied of mountain mahogany, antelope bitterbrush, and fourwing saltbush. It appears, at this time, that range of source materials comparable to the collections in the study could be used interchangeably for a variety of reclamation situations. Ecotypic differentiation with respect to competitive ability, moisture, and short growing season stress has been documented for snowberry, serviceberry, and winterfat.

The maintenance of fertility levels (especially nitrogen) on disturbed lands depends upon adequate and timely supplies of nitrogen either as fertilizer or through enhanced activity of natural elements of the ecosystems. Biological and chemical pathways have been studied as to their contribution of nitrogen to disturbed plant-soil systems.

NOTICE

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EFFECTS OF PLANT SPECIES, SOIL MATERIAL, AND CULTURAL PRACTICES UPON PLANT ESTABLISHMENT AND SUCCESSION

Edward F. Redente, Ted B. Doerr, Carolyn E. Grygiel, Erwin Allerdings,
John M. Stark, and Mario E. Biondini

OBJECTIVES

This subproject has several interrelated objectives directed toward the establishment of diverse, functional, and self-sustaining ecosystems with a minimum input of amendments. The results from this subproject will ultimately serve to provide procedures for establishing effective plant communities on lands disturbed by oil shale mining and retorting processes. Various plant species mixtures (native and introduced), planting techniques, fertilization treatments, mulch, and irrigation are being tested on disturbed soils, retorted oil shale, and soil over retorted shale material. The specific objectives of the research include: (1) determining proper seeding practices of potentially usable mixtures of plant species and their relationship to cultural practices such as fertilization, irrigation, and mulching, (2) determining the rate and direction of plant succession on disturbed topsoil, subsoil, overburden, retorted shale, and retorted shale overlain by soil as influenced by natural invasion, species mixtures, and cultural practices, and (3) determining proper management procedures for long-term stabilization and reclamation of retorted shale materials.

RESULTS TO DATE

Direct Revegetation of Paraho Retorted Oil Shale

Introduction

The objective of this study was to establish a diverse, self-sustaining plant community with a minimum of cultural inputs. Initial attempts to establish plants directly on Paraho retorted oil shale using only fertilization proved unsuccessful in 1977 (Redente et al. 1981). Therefore, in 1979 new seed mixtures and fertilizer rates were applied with straw mulch in order to establish functional plant communities.

Methods

The three seed mixtures used were composed of either all native, all introduced, or a salt-tolerant species mixture (Table 1). Three combinations of nitrogen and phosphorus were applied at (1) 56 kg N/ha, 672 kg P/ha; (2) 56 kg N/ha, 488 kg P/ha; and (3) 56 kg N/ha, 224 kg P/ha. These three rates were chosen because greenhouse studies revealed that Paraho retorted oil shale was phosphorus deficient, which severely limited plant growth (Redente, unpublished data). The phosphorus was incorporated into the upper 15 cm of the profile using a tractor-mounted rototiller. The nitrogen was applied during the spring of the first growing season following seedling emergence. Seeds were hand-broadcasted and covered by raking after the phosphorus was applied. Finally, seed-free straw mulch was applied at 2.2 MT/ha to reduce the high shale surface temperatures and to enhance available moisture for germinating seeds.

The 1981 Surface-to-Shale data was analyzed using multiple regression. Means with significant F-statistics were further evaluated using the Least Significant Difference (LSD) method. All statistical analyses were completed by species and life form (grasses, forbs, shrubs).

Results and Discussion

Generally, individual plants growing on Paraho retorted shale are low in stature and biomass, atypical in color, and lack vigor and reproductive capacities. Open spaces of the seeded communities have become dominated by invading plants, particularly Russian thistle (Salsola iberica) and kochia (Kochia scoparia).

Table 1. Seed mixtures and rates used on the Retorted Shale-to-Surface Study.

Common Name	Scientific Name	Seeding Rate (kg/ha)
Mixture A--Salt-tolerant species		
1. Jose tall wheatgrass	Agropyron elongatum	4.5
2. Rosana western wheatgrass	Agropyron smithii	2.2
3. Critana thickspike wheatgrass	Agropyron dasystachyum	1.1
4. Oahe intermediate wheatgrass	Agropyron intermedium	2.2
5. Slender wheatgrass	Agropyron trachycaulum	2.2
6. Vinal Russian wildyre	Elymus junceus	1.1
7. Madrid yellow sweetclover	Melilotus officinalis	1.1
8. Ladak alfalfa	Medicago sativa	1.1
9. Strawberry clover	Trifolium fragiferum	1.1
10. Fourwing saltbush	Atriplex canescens	4.5
11. Shadscale saltbush	Atriplex confertifolia	4.5
12. Mat saltbush	Atriplex corrugata	2.2
13. Castle Valley clover	Atriplex cuneata	3.4
14. Gardner saltbush	Atriplex gardneri	2.2
15. Winterfat	Ceratoides lanata	4.5
		37.9
Mixture B--Native species		
1. Beardless bluebunch wheatgrass	Agropyron inerme (spicatum)	2.2
2. Sodar streambank wheatgrass	Agropyron riparium	1.1
3. Rosana western wheatgrass	Agropyron smithii	2.2
4. Paloma Indian ricegrass	Oryzopsis hymenoides	1.1
5. Green needlegrass	Stipa viridula	1.1
6. Sweetvetch	Hedysarum boreale	6.7
7. Lewis flax	Linum lewisii	1.1
8. Palmer penstemon	Penstemon palmeri	0.6
9. Big sagebrush	Artemisia tridentata	0.1
10. Fourwing saltbush	Atriplex canescens	4.5
11. Curlleaf mountain mahogany	Cercocarpus ledifolius	4.5
12. Winterfat	Ceratoidies lanata	4.5
		34.2
Mixture C--Introduced species		
1. Nordan crested wheatgrass	Agropyron desertorum	1.1
2. Jose tall wheatgrass	Agropyron elongatum	4.5
3. Oahe intermediate wheatgrass	Agropyron intermedium	2.2
4. Siberian wheatgrass	Agropyron sibiricum	1.1
5. Luna pubescent wheatgrass	Agropyron trichophorum	2.2
6. Regar meadow brome	Bromus biebersteinii	2.2
7. Vinal Russian wildrye	Elymus junceus	1.1
8. Lutana cicer milkvetch	Astragalus cicer	2.2
9. Ladak alfalfa	Medicago sativa	1.1
10. Madrid yellow sweetclover	Melilotus officinalis	1.1
11. Small burnet	Sanguisorba minor	4.5
12. Siberian peashrub	Caragana arborescens	13.4
13. Russian olive	Elaeagnus angustifolia	44.8
		81.5

Effects of Seed Mixture

The total aboveground production and canopy cover of seeded species in the salt-tolerant mixture was significantly greater compared to the native or introduced seed mixtures in 1981 (Fig. 1). The seeded species that have remained a component of the stand, although of minor amounts, are intermediate wheatgrass (Agropyron intermedium), slender wheatgrass (A. trachycaulum), western wheatgrass (A. smithii), and winterfat (Ceratoides lanata).

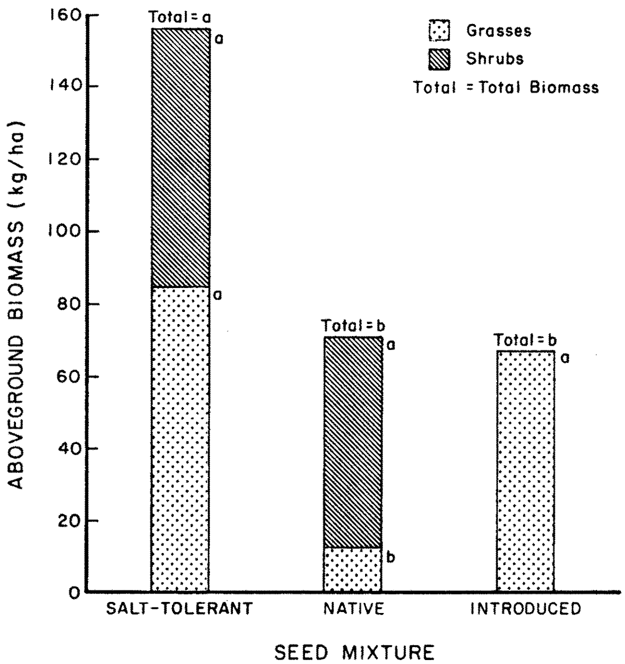

Fig. 1. Mean dry weight of aboveground seeded biomass (kg/ha) by seed mixture on the Surface-to-Shale Study in 1981. Means with different letters within life forms are significantly different (p=0.10).

The aboveground production of the seeded grasses in the salt-tolerant and introduced mixtures was significantly greater than in the native mixture (Fig. 1). Intermediate wheatgrass comprised 61% of the seeded grass aboveground biomass in the salt-tolerant mixture (52 kg/ha) and 85% (66kg/ha) in the introduced seed mixture. Western wheatgrass and streambank wheatgrass (Agropyron riparium) accounted for 91% of the seeded grass aboveground production (12 kg/ha) in the native mixture. These results indicate that intermediate wheatgrass was better adapted to the high salt content and pH values associated with Paraho retorted shale than other seeded grasses.

Seeded shrub production was similar in the salt-tolerant mixture compared to the native mixture, and production in both mixtures was significantly greater than the introduced mixture (Fig. 1). Winterfat comprised 61% and 100% of the seeded shrub biomass component in the salt-tolerant and native mixtures, respectively. Hitchcock and Cronquist (1978) described winterfat as being adapted to saline or alkaline areas. Also, winterfat will germinate readily if a viable seed source is planted (USDA 1974). The saltbushes (Atriplex spp.) accounted for only 39% of the aboveground seeded shrub production in the salt-tolerant mixture and did not germinate readily, although they have been reported as salt tolerant (USDA 1979). Medin and Ferguson (1972) included low availability of good quality seed as one of the major problems of range revegetation. Poor quality seed is one likely factor for the poor establishment of the saltbushes in this study. Siberian peashrub (Caragana arborescens) and Russian olive (Eleagnus angustifolia) failed to germinate in the introduced mixture.

Seeded forbs failed to establish in any of the three seed mixtures. Forb species such as alfalfa (Medicago sativa), yellow sweetclover (Melilotus officinalis), sweetvetch (Hedysarum boreale), Lewis flax (Linum lewisii), and cicer milkvetch (Astragalus cicer), readily found in the adjacent retorted shale topsoil study, could not withstand the harsh growing conditions of the Paraho retorted shale.

Effects of Fertilizer

Total aboveground biomass was significantly increased by the high phosphorus (P) rates (672 and 448 kg P/ha) compared to the low rate (224 kg P/ha) in 1981 (Fig. 2). This response can be explained

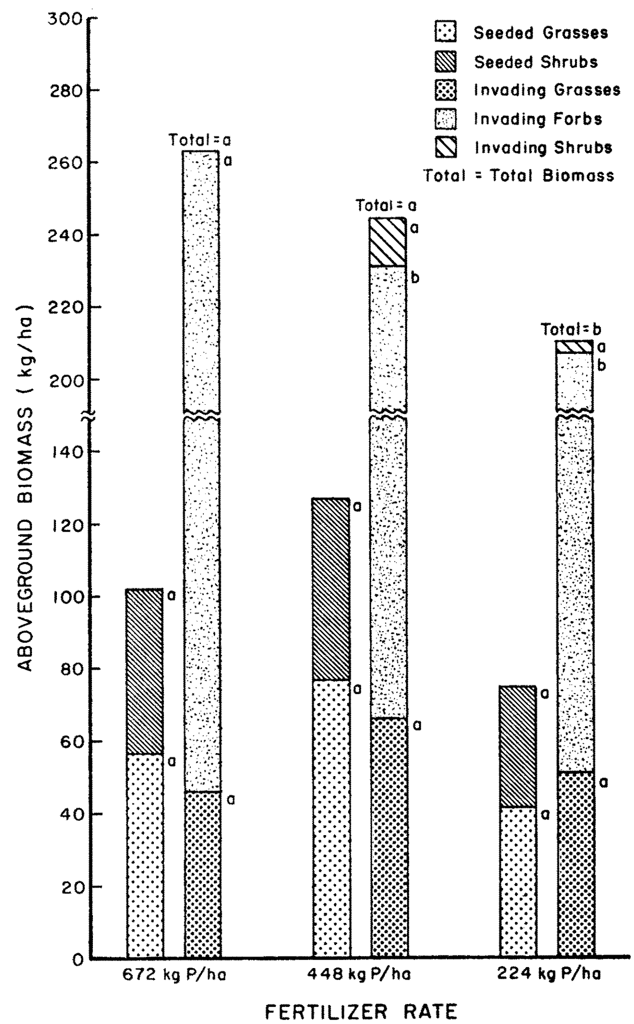

Fig. 2. Mean dry weight of aboveground biomass (kg/ha) by fertilizer rate on the Surface-to-Shale Study in 1981. Means with different letters within life form are significantly different (p=0.10).

by the significant increase of Russian thistle production where it comprised 60% (218 kg/ha) and 44% (165 kg/ha) of the total aboveground biomass on 672 kg P/ha and 448 kg P/ha treatments, respectively.

Aboveground seeded biomass was not significantly different between fertilizer rates for total production or grass, forb, or shrub production in 1981 (Fig. 2). Available phosphorus was probably not the primary limiting factor because of the high amounts added and because of the low establishment and production on the plots. High salts, sodium, pH, and surface temperatures are probably interacting and limiting germination and plant growth.

After four years of natural weathering the chemical and physical properties of Paraho retorted shale were altered only slightly according to soil test reuslts. The pH of the four-year-old retorted shale remains at 8.8, which is considered strongly alkaline and very restricting to uptake of nitrogen, phosphorus, iron, manganese, copper, and zinc (Tisdale and Nelson 1975). Electrical conductivity (EC) of the Paraho retorted shale had a saturation extract value of 18.2. A four-year period of weathering did not ameliorate the high salt content in the Paraho retorted shale. Only highly salt-tolerant plant species could grow under such saline conditions. The sodium absorption ratio dropped from initial levels of 14 in unweathered retorted shale to 9.5 after four years of weathering. Excess sodium was reduced somewhat to the stage where it wasn't as hazardous to established salt-tolerant plant species. Overall, Paraho retorted oil shale does not provide a suitable growth medium after four years of natural weathering.

Conclusions

To date adequate plant establishment has not been achieved on the Surface-to-Shale treatments. Factors such as high pH, EC, and surface temperatures have severely limited establishment and production of seeded species. Other management options need to be implemented if a successful plant community is to become established directly on Paraho retorted oil shale.

In the summer of 1981 a more intensive approach was taken to facilitate the establishment of plants directly on Paraho retorted shale. The primary approach included leaching the shale material to reduce the soluble salt level and refertilizing to provide adequate levels of plant available N and P. One-third of the area was treated with "Round-Up" (Glyphosate) to remove all existing vegetation. The vegetation on the other two-thirds of the area was left intact. Following the herbicide treatment 75 cm of water was applied to two-thirds of the entire area in order to reduce soluble salts in the rooting zone. The result, therefore, was that one-third of the area was left untreated to monitor the change in existing species composition through time and to determine the effect that Russian thistle would have on modifying the retorted shale as a plant growth medium. The second portion of the study consisted of leaving the existing plants in place but modifying the growth medium with leaching water and N and P applications. The final one-third of the area consisted of modifying the growth medium with water and fertilizer and reseeding the site to establish a new plant community using the same species mixtures that were originally used in 1979 (Table 1). The entire study will continue to be monitored in future years to determine the effectiveness of each treatment in stabilizing retorted shale and promoting a stable plant community.

Retorted Shale Successional Study

Introduction

The Retorted Shale Successional Study was initiated in the summer of 1977 to evaluate plant growth and succession as affected by various topsoil depths over retorted shale along with capillary barriers. To study this, several topsoilshale profiles were constructed to simulate conditions that may result from various retorted shale disposal plans. The dimensions of each profile treatment measured 23x109 m and varied in depth from 60 to 150 cm depending upon the treatment. The profile configurations are:

1. 30 cm topsoil over retorted shale
2. 60 cm topsoil over retorted shale
3. 90 cm topsoil over retorted shale
4. 60 cm topsoil over 30 cm rock capillary barrier over retorted shale
5. Disturbed soil control with no retorted shale (vegetation removed and soil ripped to 30 cm)

All profiles containing shale received 60 cm of Paraho (direct mode process) retorted shale from the Anvil Points retorting facility near Rifle, Colorado. The lower 15 cm of retorted shale in each profile was compacted to reduce soil water movement through the material.

Following topsoil placement the six profiles were drill seeded with three seed mixtures which consisted of diverse combinations of grasses, forbs, and shrubs. The three mixtures contained either all native, all introduced, or a combination of native and introduced species (Table 2). Nitrogen and phosphorus were applied in the following combinations:

Treatment 1: 112 kg N/ha, 56 kg P/ha

Table 2. Seed mixtures and rates used on the Retorted Shale Successional Study.

Common Name	Scientific Name	Seeding Rate (kg/ha)
Mixture A--Combination (native and introduced) species		
1. Nordan crested wheatgrass	Agropyron desertorum	1.1
2. Siberian wheatgrass	Agropyron sibiricum	1.1
3. Critana thickspike wheatgrass	Agropyron dasystachyum	1.1
4. Sodar streambank wheatgrass	Agropyron riparium	1.1
5. Slender wheatgrass	Agropyron trachycaulum	1.1
6. Regar meadow brome	Bromus biebersteinii	1.1
7. Indian ricegrass	Oryzopsis hymenoides	1.1
8. Green needlegrass	Stipa viridula	1.1
9. Durar hard fescue	Festuca ovina duriuscula	0.6
10. Madrid yellow sweetclover	Melilotus officinalis	0.6
11. Sweetvetch	Hedysarum boreale	1.1
12. Globemallow	Sphaeralcea munroana	0.6
13. Lewis flax	Linum lewisii	0.6
14. Arrowleaf balsamroot	Balsamorhiza sagittata	1.1
15. Fourwing saltbush	Atriplex canescens	1.1
16. Stansbury cliffrose	Cowania mexicana stansburiana	1.1
17. Winterfat	Ceratoides lanata	1.1
18. Green ephedra	Ephedra viridis	1.1
		17.8
Mixture B--Native species		
1. Rosana western wheatgrass	Agropyron smithii	1.1
2. Sodar streambank wheatgrass	Agropyron riparium	1.1
3. Bearded bluebunch wheatgrass	Agropyron inerme (spicatum)	1.1
4. Indian ricegrass	Oryzopsis hymenoides	1.1
5. Green needlegrass	Stipa viridula	1.1
6. Shermans big bluegrass	Poa ampla	1.1
7. Alkali sacaton	Sporobolus airoides	0.6
8. Globemallow	Sphaeralcea munroana	0.6
9. Sweetvetch	Hedysarum boreale	1.1
10. Palmer penstemon	Penstemon palmeri	0.6
11. Stansbury cliffrose	Cowania mexicana stansburiana	2.2
12. Green ephedra	Ephedra viridis	1.1
13. Fourwing saltbush	Atriplex canescens	1.1
14. Winterfat	Ceratoides lanata	1.1
15. Antelope bitterbrush	Purshia tridentata	1.1
		16.1
Mixture C--Introduced species		
1. Nordan crested wheatgrass	Agropyron desertorum	1.1
2. Siberian wheatgrass	Agropyron sibiricum	1.1
3. Jose tall wheatgrass	Agropyron elongatum	1.1
4. Luna pubescent wheatgrass	Agropyron trichophorum	1.1
5. Oahe intermediate wheatgrass	Agropyron intermedium	1.1
6. Manchar smooth brome	Bromus inermis	1.1
7. Regar meadow brome	Bromus biebersteinii	1.1
8. Vinal Russian wildrye	Elymus junceus	1.1
9. Ladak alfalfa	Medicago sativa	0.6
10. Madrid yellow sweetclover	Melilotus officinalis	0.6
11. Lutana cicer milkvetch	Astragalus cicer	0.6
12. Sainfoin	Onobrychis viciaefolia	0.6
13. Bouncing bet	Saponaria officinalis	1.1
14. Small burnet	Sanguisorba minor	2.2
15. Siberian peashrub	Caragana arborescens	1.1
16. Russian olive	Elaeagnus angustifolia	2.2
		17.8

Treatment 2: 56 kg N/ha, 28 kg P/ha

Treatment 3: no fertilizer

The study was arranged in a randomized block design with all possible treatment combinations found in each of three replications. Vegetation data was analyzed by life form (grasses, forbs, and shrubs) using multiple regression techniques to incorporate environmental factors, treatments, and interactions. Vegetation was sampled using six permanent 0.25 m^2 quadrats and two 6 m^2 belt transects per plot. Density, aboveground biomass, and percent canopy cover for all species were recorded using nondestructive estimation techniques. Number and percent cover of seeded forb and shrub species, generally under-sampled using the quadrat method, were recorded in each belt transect to supplement quadrat data. Subsurface soil moisture was recorded the first of May through September at 15-cm increments to a depth of 90 cm (except on the 60 cm soil over 30 cm capillary barrier profile which was sampled to a depth of 60 cm to maintain the integrity of the capillary barrier).

Results and Discussion

Total precipitation between November 1980 and April 1981 was 63.5 mm, 88.9 mm below the average. Therefore, the low available soil moisture probably stressed plants, resulting in lower vegetation production levels in 1981 compared to 1980.

Effects of Topsoil Depth Over Retorted Shale

Aboveground production of seeded species was greater on the 90-cm soil treatment and 60-cm soil/capillary barrier treatment compared to the shallower soil treatments and the soil control in 1981 (Fig. 3). The 60-cm topsoil/capillary barrier treatment supported significantly more total aboveground biomass than all other treatments including 90-cm soil over shale. Overall, aboveground biomass on the 30-cm soil over shale and soil control were the lowest of all treatments studied. The overriding factor deciding the potential success of revegetation attempts in a semiarid environment is the availability of water (Thornberg and Fuchs 1978). The 30-cm gravel layer in the 60-cm soil/capillary barrier treatment produced a temporarily perched water table because of the sharp gradient in pore size between the soil and the gravel layer. This artificial enhancement of the effective water available for plant growth was demonstrated by the significantly greater amount of total biomass produced in this treatment. The Paraho retorted oil shale inhibited root penetration. This was indicated by the shallow penetration of roots past the soil/shale interface. Therefore, water and nutrient uptake of the plant species were restricted mainly to the overlying topsoil layer. The increasing soil volumes from the 30 to 90-cm soil treatments provided a larger volume of soil from which both water and nutrients could be extracted by the plant community.

In 1981, seeded grass production was highest on the 60-cm soil/capillary barrier treatment

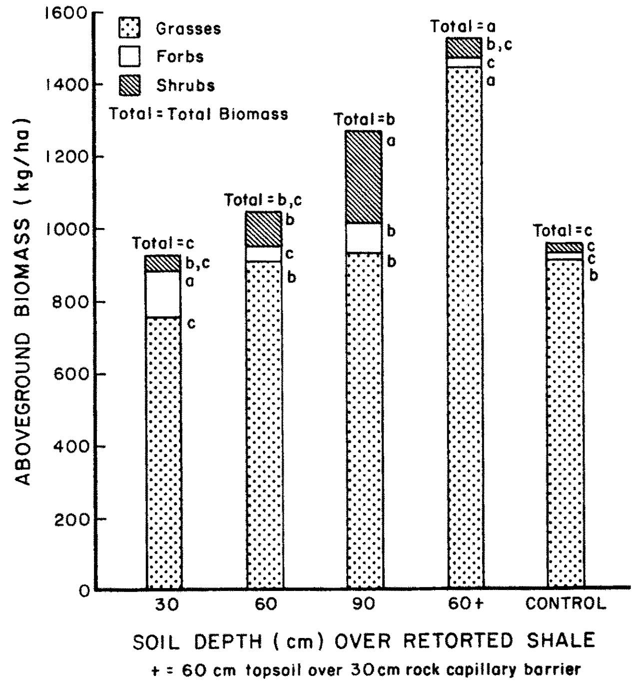

Fig. 3. Mean dry weight of aboveground seeded biomass (kg/ha) by soil treatment on the Retorted Shale-Topsoil Study in 1981. Means with different letters within life forms are significantly different (p=0.10).

compared to all other treatments including the control (Fig. 3). Seeded grass biomass on the 60- and 90-cm soil thickness treatments and the control were not significantly different. Grass biomass on the 30-cm soil treatment was significantly less compared to all other treatments.

Seeded forb biomass was significantly higher on the 30-cm soil treatment compared to all other treatments in 1981 (Fig. 3). Seeded forb biomass was significantly higher on the 90-cm topsoil covering compared to the 60-cm, 60-cm/capillary barrier, and control treatments which had similar forb production. The seeded grass roots dominated the larger soil volumes and the 60-cm soil/capillary barrier treatment efficiently and quickly enough to tie up much of the resources the seeded forbs required for establishment and growth. Also, the grasses further restricted forb establishment by closure of the plant stand through shading, litter accumulations, and space preemption. The smaller rooting volume of the 30-cm soil treatment may have helped the seeded forbs in competing for the essential resources by limiting growth of the seeded grasses. Less subsequent limitations on the establishment of new forbs are also shown by the significantly higher seedling densities of such species as alfalfa and yellow sweetclover.

Shrub biomass was significantly higher on the 90-cm soil depth compared to all other treatments and significantly higher on the 60-cm soil depth compared to the control treatment in 1981. The 30-cm and 60-cm soil/capillary barrier treatments were not significantly different from the 60-cm or control treatments (Fig. 3). The rooting morphology of the shrubs allow these species to reach and absorb moisture at deeper soil depths compared to grasses (Stoddart et al. 1975). The deeper soil depths in the 90-cm soil treatment may have been vacant long enough for the seeded shrub roots to initiate use of available resources before the grass roots could. Root systems of the slower establishing seeded shrub species, green ephedra (Ephedra viridis) and bitterbrush (Purshia tridentata), were unable to grow rapidly enough to avoid competition for moisture with the aggressive grasses. Low shrub production on the control treatment may be partially due to a calcium carbonate layer at the 28 to 36 cm range that would impede root development at deeper depths and limit deep soil moisture.

Effects of Seed Mixture

The native and combination seed mixtures produced significantly more total aboveground biomass than the introduced mixture in 1981 (Fig. 4). The combination seed mixture did not have a significantly different total seeded biomass compared to the native mixture. Overall, the native species accounted for a greater biomass production than the introduced species in the combination mixture. Therefore, native species may be more competitive

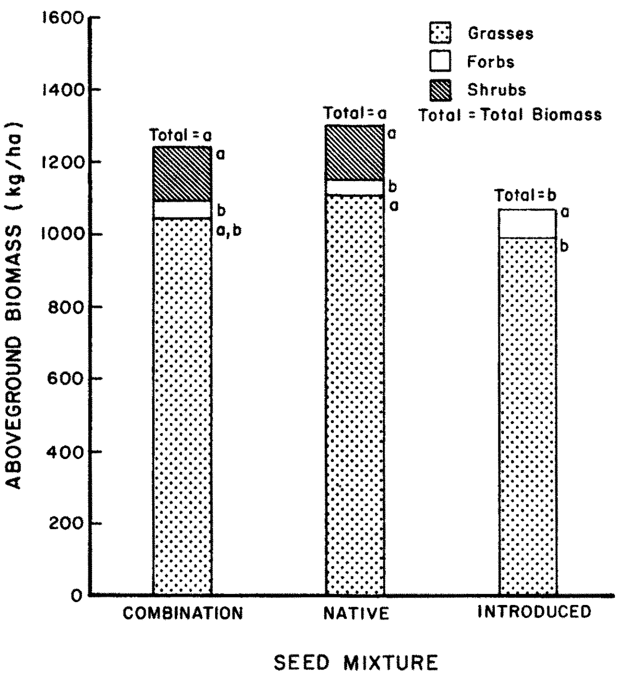

Fig. 4. Mean dry weight of aboveground seed biomass (kg/ha) by seed mixture on the Retorted Shale-Topsoil Study in 1981. Means with different letters within life forms are significantly different (p=0.10).

than the introduced species under the wide range of environmental factors on this site.

The native seed mixture had significantly greater seeded grass production than the introduced mixture (Fig. 4). Seeded grass biomass in the combination mixture was not significantly different from production in either the native or introduced mixtures. Crested wheatgrass (Agropyron desertorum) and pubescent wheatgrass (A. trichophorum) were the dominant introduced species composing 34% and 30% of the biomass, respectively. Big bluegrass (Poa ampla) and the streambank-thickspike wheatgrass (Agropyron dasystachyum) complex were the dominant native grass species composing 38% and 26% of the biomass, respectively. These dominant species generally have good seedling vigor, virtually no germination inhibitions, and rapid growth rates in the environment being studied. Meadow brome (Bromus biebersteinii) was one of the grasses least adapted to the semiarid conditions of the study site and, therefore, contributed little to the overall composition of the established mixtures. Big bluegrass generally has poor germination or seedling vigor in semiarid environments. However, precipitation the winter and spring before the first growing season was above average. Therefore, an atypically mesic environment was present to allow good early growth of big bluegrass. Grass species that did not compose a large percent of the plant composition included species requiring more mesic environments (smooth brome (B. inermis)), species that have poor germination or weak seedling vigor (green needlegrass (Stipa viridula), Indian ricegrass (Oryzopsis hymenoides) and Russian wildrye (Elymus junceus)), and species that are shortlived (slender wheatgrass and tall wheatgrass (Agropyron elongatum)).

Seeded forb production was significantly greater in the introduced mixture compared to the native and combination mixtures in 1981 (Fig. 4). Alfalfa comprised 78% of the seeded forb biomass in the introduced mixture. Sainfoin (Onobrychis viciaefolia), yellow sweetclover, and cicer milkvetch were other established forbs in the introduced mixture. Sweetvetch provided 80% of the seeded forb biomass in the native mixture. Palmer penstemon (Penstemon palmeri) and globemallow (Sphaeralcea munroana) composed the remaining 20%. Sweetvetch, Lewis flax, and arrowleaf balsamroot (Balsamhoriza sagittata) composed 48%, 31%, and 12%, respectively, of the seeded forb biomass in the combination mixture. Alfalfa and sweetvetch were the two species that have maintained densities greater than 1 plant/m^2 (unlike globemallow, milkvetch, and sainfoin) and have a potential to produce a large amount of biomass (unlike arrowleaf balsamroot, Lewis flax, and Palmer penstemon). Therefore, forb composition (calculated by biomass) was dominated by alfalfa and sweetvetch. Forb composition calculated by density still showed that these two species were the dominant forbs. However, sweetclover, arrowleaf balsamroot, Lewis flax, and Palmer penstemon each contributed at least 20% of forb biomass in the seed mixtures they were found in. Therefore, these species also should be considered successful reclamation species in this study.

The introduced shrubs, Siberian peashrub, and Russian olive, did not become established in the introduced mixture (Fig. 4). Environmental conditions at the time of seeding, the lack of preseeding treatment of the seed, competition from rapidly establishing grasses, or a combination of these factors probably hindered the establishment of these two shrubs. Fourwing saltbush (Atriplex canescens) and winterfat were the primary shrub species, collectively contributing 97% and 98% of seeded shrub production in the native and combination mixtures, respectively. Fourwing saltbush and winterfat each comprised 50% of shrub production in the combination mixture; however, fourwing saltbush production was three and one-half times greater than winterfat production in the native mixture. This difference was accounted for by the doubling of winterfat densities and aboveground biomass from the native to the combination seed mixture, while the densities and biomass production of fourwing saltbush remained basically similar between the two treatments.

Effects of Fertilizer

Following the fourth growing season (1981), the response to nitrogen (N) and phosphorus (P) was still evident. Total seeded biomass was greatest on the high fertilizer treatment (112 kg N/ha, 56 kg P/ha) (Fig. 5). Total aboveground biomass on the moderate fertilizer treatment (56 kg N/ha, 28 kg P/ha) was similar to production on the control after four years.

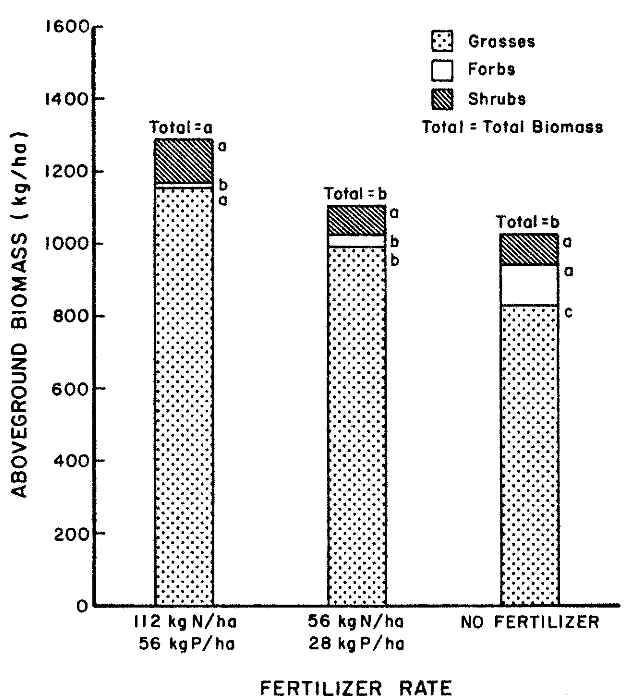

Fig. 5. Mean dry weight of aboveground seeded biomass (kg/ha) on the Retorted Shale-Topsoil Study in 1981. Means with different letters within life forms are significantly different (p=0.10).

Seeded grass production increased with increasing fertilizer rates and accounted for the overall fertilizer response in the study (Fig. 5). This is because grass species respond to nitrogen fertilizer inputs in the upper soil profile with greater efficiency compared to other life forms (Wight and Black 1978). Crested wheatgrass, pubescent wheatgrass, and intermediate wheatgrass exhibited the greatest gains from increased N and P applications.

Seeded forb biomass decreased with an increase in fertilizer rates (Fig. 5). Essential plant nutrients, such as N and P, may have been less available to the seeded forbs because the grass species effectively out competed forbs for N, P, moisture, and space.

Shrub production did not vary significantly across fertilizer treatments (Fig. 5). Woodmansee et al. (1978) found that some shrubs grew well on sites with an N deficiency. The N requirements of the woody plants were being met by the internal cycling and subsequent conservation of the nutrient. This translocation process within the shrubs has been estimated to satisfy 60% or more of the N requirements during growth (Charley 1977). Therefore, shrub species do not rely as heavily on available N content in the profile as the grass species do, subsequently no significant response has been measured.

Conclusions

The following conclusions are based on fourth-year data and may be subject to change as the established plant communities respond to inter- and intraspecific competition and environmental fluctuations.

The 90-cm topsoil and 60-cm topsoil/capillary barrier treatments supported the greatest amount of plant growth. The 60-cm topsoil/capillary barrier treatment is the most productive at present and will likely continue to be until such time as the integrity of the capillary barrier is sufficienty reduced. At this point, salt movement upwards and loss of perched water above the capillary barrier may combine to adversely affect productivity of the plant communities.

Seeded grasses were most dominant in the capillary barrier treatment. However, seeded shrubs appeared to increase with soil depth and the greatest aboveground production was recorded on the 90-cm topsoil treatment. The alteration of topsoil depths and presence of a capillary barrier are directly affecting overall species composition and life form dominance. The manipulation of topsoil depth and use of capillary barriers may ultimately prove useful as a management tool in modifying the eventual plant community expression.

Total aboveground production was greater for native species compared to introduced species. This is possibly an indication of the long-term biologic potential and adaptability to the environmental conditions of native species at the study site. Increasing rates of N and P increased the dominance of grass species and decreased the productivity of forbs. Introduced grass species such as crested wheatgrass, pubescent wheatgrass, and intermediate wheatgrass appear to be directly responding to increased soil fertility. Seeded forbs such as sweetvetch and alfalfa are more competitive with other species at lower fertility levels. These results indicated that fertilizer can be used to control individual species responses and alter short-term plant community expression.

Annual Disturbance Study

Introduction

The Annual Disturbance Study dealing with the disturbance of a new area each year was initiated in 1976 to determine the effects of soil disturbance and annual weather variations on natural plant invasion and succession. Soil was disturbed at four levels of intensity for three years to simulate various degrees of disturbance which may accompany oil shale development. The disturbance treatments were:

Treatment 1: Vegetation was mechanically removed and the topsoil was left in place.

Treatment 2: Vegetation was mechanically removed and the soil was scarified to a depth of 30 cm.

Treatment 3: Vegetation was mechanically removed and 1 m of soil (A, B, and C horizons) was removed, mixed together, and returned to the excavated area.

Treatment 4: Vegetation was mechanically removed. The top 1 m of soil was removed and stockpiled. A second meter of soil was removed and stockpiled. The first meter of soil was placed in the excavated area, and the second meter of soil was placed over the first meter of soil.

The study was a randomized block design with year of disturbance representing the variation being removed by the block. Two replicates of each soil treatment were established annually. After disturbance vegetation was sampled each year in July (representing peak aboveground production) by using ten 0.25-m^2 permanent quadrats randomly located in each treatment plot. Density, aboveground biomass, and percent canopy cover were recorded by species using nondestructive estimation techniques. All data presented herein were collected in July of 1981 from plots initially disturbed in 1976, 1977, and 1979. Data were analyzed using analysis of variance techniques, and means were separated at the 90% confidence level.

Results and Discussion

Effect of Treatment

Treatment 1 had significantly greater grass production compared to Treatments 2, 3, and 4 and Treatment 2 had significantly greater grass biomass than Treatment 4 (Fig. 6). The major grass species inhabiting the area were western wheatgrass and streambank wheatgrass. These species comprised 43% of the composition in Treatment 1 plots and 20% on Treatment 2 plots (Table 3). The primary grass species on Treatment 3 were prairie junegrass (Koleria cristata) and Indian ricegrass. The composition shift from wheatgrasses to Indian ricegrass exhibited in Treatment 3 may be due to the successional status of this species. Western and streambank wheatgrasses are generally found in later successional stages of the mid-elevation sagebrush type. Indian ricegrass, on the other hand, is found as an early successional species on disturbed sites, especially on sandy soils. Also, wheatgrass seed and rhizome sources were better maintained in the upper horizons of Treatment 1 compared to Treatments 2 and 3 because of the comparatively light soil disturbance. Therefore, wheatgrasses recovered more rapidly in Treatment 1, and to some extent in Treatment 2, precluding high establishment of prairie junegrass and Indian ricegrass. Conversely, deeper soil disturbance in Treatment 3 allowed greater establishment of Indian

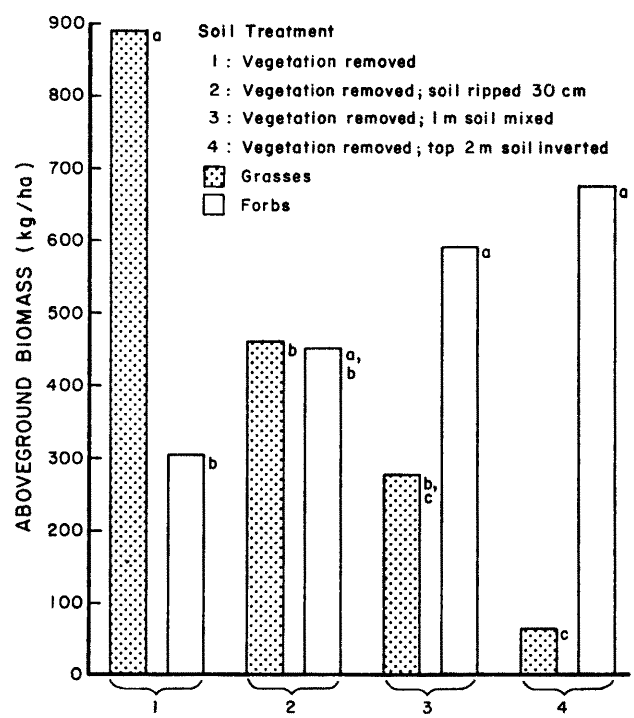

Fig. 6. Mean dry weight of aboveground biomass of grasses and forbs on 1976, 1977, and 1979 disturbance treatments in 1981. Life forms with different letters are significantly different (p=0.10).

Table 3. Percent composition in 1981 of major grass species and Russian thistle in each soil treatment in the Annual Disturbance Study for 1976, 1977, and 1979 disturbance plots, combined.

Disturbance Treatment	Russian thistle	Streambank wheatgrass	Western wheatgrass	Prairie junegrass	Indian ricegrass	Total Grasses
1	7	23	20	7	0	50
2	21	16	4	0	7	27
3	63	3	2	5	11	30
4	62	0	0	0	0	0

ricegrass and junegrass. In addition, Indian ricegrass and junegrass may not be as dependent on mycorrhizae as wheatgrasses (Reeves, personal communication). This would favor the establishment of Indian ricegrass compared to wheatgrasses on Treatment 3 plots because of the lower Mycorrhizal Inoculum Potential (MIP) values found on Treatment 3 plots compared to Treatment 1 plots.

Forb production was significantly greater in Treatments 3 and 4 compared to Treatment 1 (Fig. 6). Forb biomass in Treatment 2 was slightly higher than Treatment 1 but was not significantly different than biomass on Treatments 1, 3, or 4. Russian thistle composed 80% of forb production in 1981 and was the dominant species on Treatment 3 and 4 plots (Table 3). Its dominance in Treatments 3 and 4 can be attributed to its ability to set seed in large quantities and the ability of the seed to germinate under a wide variety of conditions and grow rapidly with a minimum of water, thereby outcompeting grass seedlings. Also, Russian thistle does not have a symbiotic relationship with mycorrhizae. Therefore, low MIP levels in Treatments 3 and 4 do not directly affect Russian thistle establishment and production as they do most perennial grasses.

Shrub density, biomass, and canopy cover have been reduced by all disturbance treatments compared to native vegetation. Rabbitbrush (Chrysothamnus spp.), big sagebrush (Artemisia tridentata), and perennial broom snakeweed (Gutierrezia sarothrae) were the three shrub species to reestablish. Shrub densities ranged from 0.4 to 3.1 plants/m^2, biomass ranged from 2.3 to 18.0 kg/ha, and canopy cover ranged from 0.1 to 1.2%. In comparison, the shrub component of undisturbed vegetation had a density of 3.5 plants/m^2, biomass of 284 kg/ha, and canopy cover of 14.1%. Competition with perennial grasses and annual forbs and few microsites suitable for shrub establishment where forb and grass competition is low (mainly on highly disturbed sites due to a high surface rockiness) have probably combined to reduce successful shrub establishment.

Effect of Year of Disturbance

Differences in biomass and cover of grasses, forbs, and total vegetation were seen among years of disturbance. Generally, grass, forb, and total vegetation biomass and canopy cover was greatest on 1976 disturbed plots, intermediate on 1977 disturbed plots, and lowest on 1979 disturbed plots in 1981 (Fig. 7). Grass canopy cover was virtually identical on 1977 plots (2.6%) and 1979 plots (2.7%) in 1981 even though 1977 plots had two extra growing seasons for succession to occur. The slower ecesis of perennial vegetation on 1977 plots may be a reflection of the lower moisture conditions prevailing at the time of disturbance compared to 1976 and 1979 moisture conditions. However, the amount of time for plant occupancy appears to supercede the effect of year of disturbance as demonstrated by the increasing total production trend from a low on 1979 plots to the high on 1976 plots.

Year of disturbance did not significantly affect shrub production . However, shrub establishment and production appear to increase as the

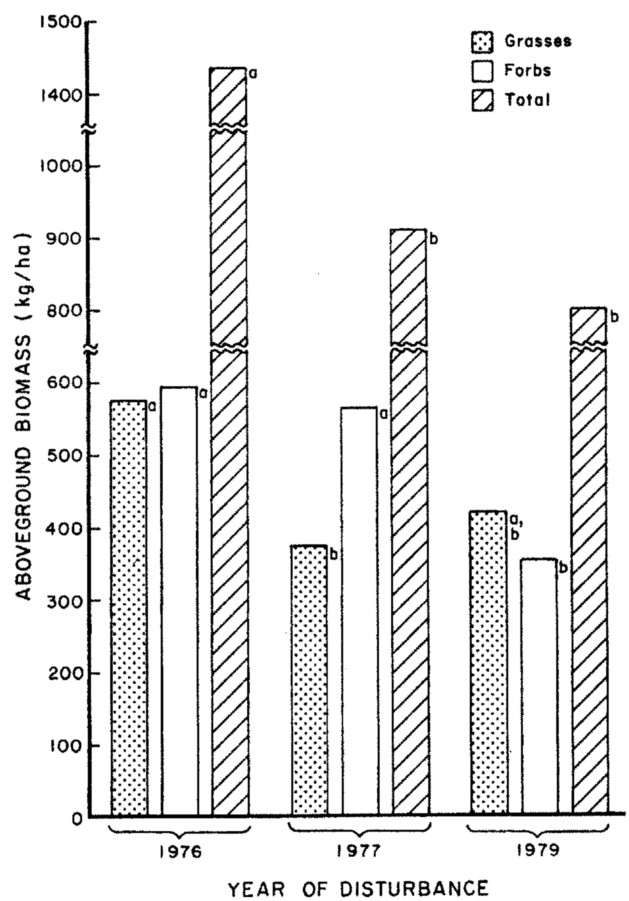

Fig. 7. Mean dry weight of aboveground biomass of grasses, forbs, and total vegetation averaged over all disturbance levels in 1976, 1977, and 1979 disturbance treatments. Life forms with different letters are significantly different (p= 0.10).

plots age. Shrub densities increased from 1 plant/m^2 on 1979 plots to 2 plants/m^2 on 1977 plots to 4.5 plants/m^2 on 1976 plots. Similarly, production was least on 1979 plots (2.4 kg/ha), intermediate on 1977 disturbed plots (7.6 kg/ha), and greatest on 1976 disturbed plots (26.3 kg/ha).

Conclusions

The effect of disturbance treatments indicates that the way soil is handled may substantially influence the amount and type of natural plant invasion that can occur on mined sites. Mixing soil horizons reduces the probability that perennial species would contribute significantly to rapid invasion and increases the probability that annual weeds would be the more prominent invaders. There is also some indication that natural recovery of disturbed soils may be retarded when the disturbance is done under dry climatic periods. This phenomenon however may be only temporary. If disturbance is done under dry conditions and these conditions prevail for several years after disturbance, then successional development will probably be severely retarded.

Surface Disturbed Successional Study

Introduction

This successional study was established in June of 1976 to determine the effects of seed mixture, fertilizer, and mulch on succession of seeded communities on surface disturbed plots. Emphasis was placed on achieving maximum production, cover, and diversity of seeded species while suppressing invasion by using minimum cultural inputs.

Site preparation consisted of scraping off the existing vegetation and scarifying the soil to a depth of 30 cm to simulate minor disturbances which may result from construction of support facilities and utility corridors. The site was then seeded with six separate seed mixtures ranging from a simple grass mixture to a complex grass-forb-shrub mixture including both native and introduced species (Table 4). Two levels of N and P fertilizer were applied at the following rates: 112 kg N/ha and 56 kg P/ha, 56 kg N/ha and 28 kg P/ha, and a control. The P was applied prior to seeding, and the N was applied following the first growing season in an attempt to reduce competition from annual invading species. Wood fiber hydromulch was applied to one-half of the plots at the rate of 2.2 MT/ha following seeding in the fall of 1976.

The study was a split-split-block design consisting of three replications and a total of 108 subplots, measuring 9x18 m. Ten 0.25 m^2 permanent quadrats were randomly placed in each subplot. Density and percent canopy cover of seeded and invading species were ocularly estimated for each quadrat. Aboveground biomass was obtained by using nondestructive estimation techniques during peak vegetation production.

Multiple regression techniques were used to isolate effects of treatments, environmental elements, and their interactions. Least significant difference (LSD) mean separation test was used to determine significant differences from the control plots. Also, percent soil moisture was used as a covariate to remove interaction effects between soil moisture and treatments.

In 1980, a decision was made to sample this study every third growing season beginning in 1981. This decision was based upon the observation that differences in biomass, density, and cover for the various seeding mixtures from year to year was becoming less pronounced as the communities matured.

Results and Discussion

Effects of Seed Mixtures

Total aboveground production in 1979 for the seeded species was greatest in the introduced grass mixture, the native grass-forb-shrub, and combination native and introduced grass-forb-shrub mixtures and lowest in the native grass, native grass-forb, and introduced grass-forb mixtures (Fig 8.). These trends were caused primarily by grass production where grasses composed 89-100% of species production except in the native grass-forb-shrub mixture where shrubs composed 65% and grasses 33% of the composition. Forbs composed less than 4% composition in any mixture.

In 1981 there was a general reduction in total production (Fig. 9) compared to 1979 because of the generally low moisture during the winter, spring, and summer of 1981. However, introduced grass and introduced grass-forb mixtures had greater total production compared to all other mixtures.

Grasses

Seeded grass biomass was significantly greater in the introduced grass mixture and the combination grass-forb-shrub mixtures compared to seeded grass production in all other mixtures in 1979 (Fig 8). There was a substantial reduction in grass biomass in 1981 compared to 1979 grass biomass levels in all mixtures except the introduced grass-forb mixture (Figs. 8 and 9). The introduced grass mixture compared to the native grass mixture showed a greater decline in production from 1979 to 1981. Readings on these plots in the future will determine the true capabilities of introduced species to withstand the vagaries of the environment compared to native species. Seeded grass biomass was significantly lower in the native grass-forb-shrub mixture compared to all other mixtures. This is probably caused by competition for space and moisture with the native shrubs (fourwing saltbush and winterfat).

Russian wildrye was the dominant introduced grass species, composing at least 40% of the

Table 4. Mixtures seeded during November 1976 on the Surface Disturbed Successional Study Area.

Common Name	Scientific Name	Seeding Rate (kg/ha)
Mixture A--Native grass mixture		
1. Bearded bluebunch wheatgrass	Agropyron spicatum	3.4
2. Rosana western wheatgrass	Agropyron smithii	4.5
3. Green needlegrass	Stipa viridula	3.4
4. Indian ricegrass	Oryzopsis hymenoides	2.2
5. Sodar streambank wheatgrass	Agropyron riparium	3.4
Mixture B--Introduced grass mixture		
1. Nordan crested wheatgrass	Agropyron desertorum	3.4
2. Luna pubescent wheatgrass	Agropyron trichophorum	4.5
3. Vinal Russian wildrye	Elymus junceus	3.4
4. Oahe intermediate wheatgrass	Agropyron intermedium	4.5
Mixture C--Native grass-forb mixture		
1. Critana thickspike wheatgrass	Agropyron dasystachyum	3.4
2. Green needlegrass	Stipa viridula	2.2
3. Bearded bluebunch wheatgrass	Agropyron spicatum	2.2
4. Indian ricegrass	Oryzopsis hymenoides	1.1
5. Sodar streambank wheatgrass	Agropyron riparium	2.2
6. Sweetvetch	Hedysarum boreale	1.1
7. Emerald crownvetch	Coronilla varia	1.1
8. Lewis flax	Linum lewisii	1.1
9. Palmer penstemon	Penstemon palmeri	1.1
Mixture D--Introduced grass-forb mixture		
1. Vinal Russian wildrye	Elymus junceus	3.4
2. Nordan crested wheatgrass	Agropyron desertorum	3.4
3. Luna pubescent wheatgrass	Agropyron trichorphorum	3.4
4. Ladak alfalfa	Medicago sativa	1.1
5. Bouncing bet	Saponaria officinalis	1.1
6. Small burnet	Sanguisorba minor	1.1
7. Lutana cicer milkvetch	Astragalus cicer	2.2
Mixture E--Native grass-forb-shrub mixture		
1. Indian ricegrass	Oryzopsis hymenoides	2.2
2. Bearded bluebunch wheatgrass	Agropyron spicatum	2.2
3. Rosana western wheatgrass	Agropyron smithii	4.5
4. Emerald crownvetch	Coronilla varia	1.1
5. Sweetvetch	Hedysarum boreale	1.1
6. Stansbury cliffrose	Cowania mexicana stansburiana	1.1
7. Green ephedra	Ephedra viridis	1.1
8. Fourwing saltbush	Atriplex canescens	2.2
9. Winterfat	Ceratoides lanata	1.1
Mixture F--Native and introduced grass-forb-shrub mixture		
1. Green needlegrass	Stipa viridula	2.2
2. Bearded bluebunch wheatgrass	Agropyron spicatum	2.2
3. Nordan crested wheatgrass	Agropyron cristatum	2.2
4. Luna pubescent wheatgrass	Agropyron trichophorum	2.2
5. Lutan cicer milkvetch	Astragalus cicer	1.1
6. Sweetvetch	Hedysarum boreale	1.1
7. Stansbury cliffrose	Cowania mexicana stansburiana	1.1
8. Green ephedra	Ephedra viridis	2.2
9. Winterfat	Ceratoides lanata	1.1

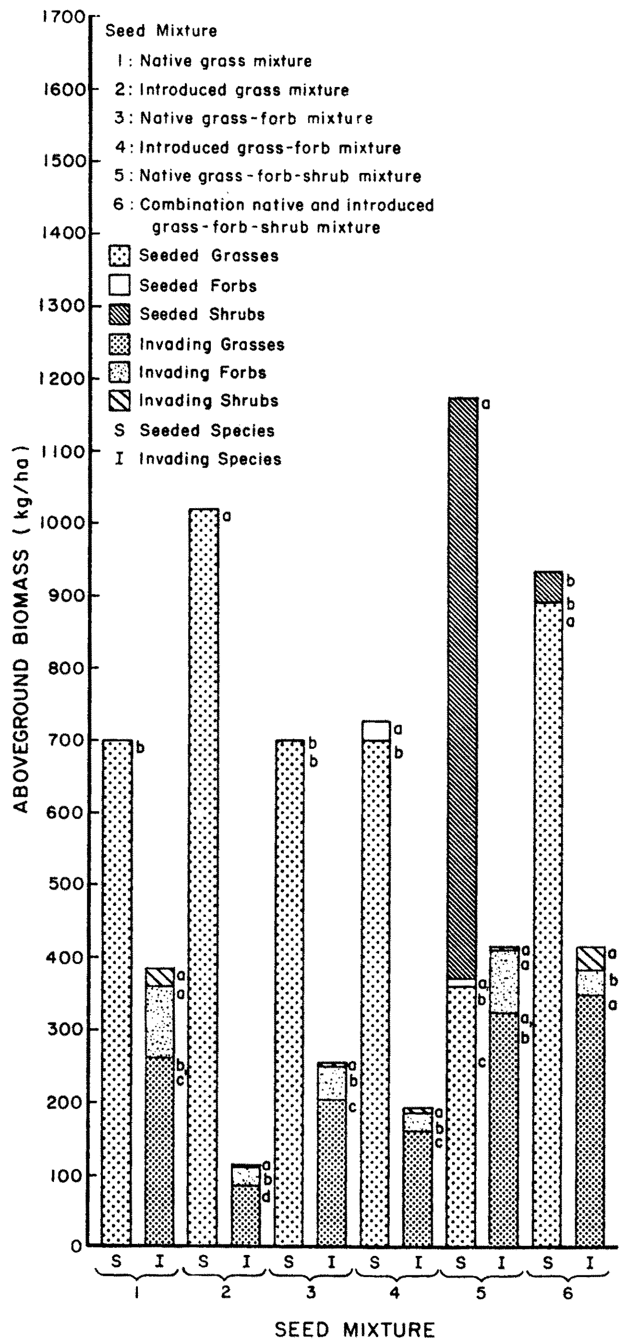

Fig. 8. Mean dry weight of aboveground biomass of seeded species and invading species in 1979. Means with different letters within life form are significantly different (p=0.10).

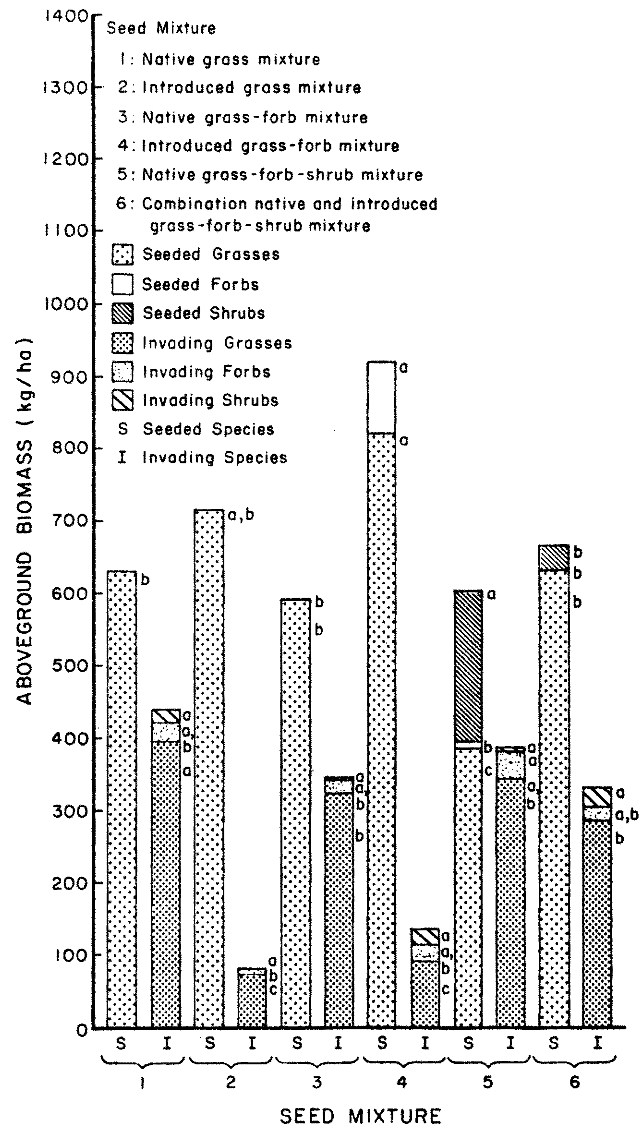

Fig. 9. Mean dry weight of aboveground biomass of seeded and invading species in 1981. Means with different letters within life form are significantly different (p=0.10).

biomass in the introduced species mixtures in 1979 and 1981. This species is persistent, but as reported by Vallentine et al. (1971), does not always regenerate well within a stand. This was evident by the slight decline in density but relatively stable biomass of 225 kg/ha in 1979 and 260 kg/ha in 1981.

Pubescent wheatgrass exhibited the greatest biomass and cover increases in the introduced grass mixture between the growing seasons of 1979 and 1981. Pubescent wheatgrass is considered to be a highly competitive, drought resistant species (Smoliak and Johnston 1975) and will tend to dominate (Plummer et al. 1968) the less drought tolerant intermediate wheatgrass when planted in the same seed mixture. Intermediate wheatgrass production declined in 1981 compared to 1979 production possibly because of competition with Russian wildrye and pubescent wheatgrass.

Crested wheatgrass production was less in 1979 than 1981, but canopy cover was relatively stable during this time. Crested wheatgrass still composed at least 20% of the biomass from introduced species mixtures in 1981. Hull (1971, 1974) and Vallentine (1971) found the species to be long-lived and predominant in seeded stands. McWilliams and Van Cleave (1960), however, found crested wheatgrass unable to resist invasion from needle-andthread grass (Stipa comata) in Montana, and Berg and Barrau (1973) in Colorado reported a decline in the plant when it was shaded by taller wheatgrasses. The decline in aboveground biomass in our study may be partially a result of shading from other grasses such as Russian wildrye and pubescent wheatgrass.

Native grass species composition has changed between 1979 and 1981 in all mixtures where they appear. The streambank-thickspike wheatgrass complex was the dominant native component in 1979 but declined to become a subdominant in 1981. Beardless bluebunch wheatgrass (Agropyron inerme) became the dominant native grass species in 1981 as it increased in production and streambank-thickspike wheatgrasses declined. Aboveground biomass from green needlegrass increased between 1979 and 1981, while exhibiting no significant increase in density. This indicates that the mature plants are established and have successfully maintained themselves in the native grass mixture. Indian ricegrass biomass declined between 1979 and 1981. Although it is a drought-resistant, long-lived perennial species (Verner 1956), it is not easily established (Pearson 1979) nor is it a vigorous competitor during early establishment. Therefore, the decline in production between 1979 and 1981 probably was a result of low vigor of the individual seedlings that were overcome by more aggressive wheatgrasses and green needlegrasse for moisture.

Forbs

Seeded forb production was comparatively low but was significantly greater in the introduced grass-forb mixture than in all other mixtures in 1979 and 1981 (Figs. 8 and 9). This resulted primarily because the more aggressive introduced forb species seeded in this mixture occupied the available space rather quickly compared to the less aggressive native forb species.

Alfalfa and small burnet (Sanguisorba minor) respectively were the dominant and subdominant forb species found in the introduced grass-forb mixture. Alfalfa is a competitive dry land species capable of rapid growth (Vallentine 1971), and once established it is rather drought resistant. Small burnet is a short-lived forb that will establish well (Plummer et al. 1968) on arid foothill ranges. However, Plummer et al. (1968) also reported that this species does not satisfactorily reestablish from natural seed production. Therefore, alfalfa may be more suitable for long-term maintenance and production compared with small burnet. Small burnet may continue to show a biomass increase from individual plants; however, a gradual decline is expected over the next several years.

Another introduced forb, cicer milkvetch, has been shown to be slow to establish (Block 1968). Therefore, it probably was competitively reduced by the more vigorous grass seedlings. However it has been successfully seeded in other revegetation studies in the Piceance Basin including the Revegetation Techniques Study. Slight differences in seeding rates and soil media probably combined to produce the differences in success of this species.

Sweetvetch was the most successful native forb species to establish and survive for five growing seasons. However, densities and aboveground production were low in all seed mixtures in which it was seeded.

Shrubs

Aboveground shrub biomass was significantly greater in the native grass-forb-shrub mixture compared to the combination native and introduced grass-forb-shrub mixture in 1979 and 1981 (Figs. 8 and 9). This was primarily the result of the presence of fourwing saltbush in the native grass-forb-shrub mixture and not in the combination native and introduced grass-forb-shrub mixture. Fourwing saltbush was the dominant species in the native grass-forb-shrub mixture. McArthur et al. (1974) found that this species is often the dominant species in communities where it occurs. The large decline in biomass in 1981 compared to 1979 was caused by less production per plant because of low moisture levels in 1981 and not due to the loss of individual plants of the species.

Effect of Fertilizer

Biomass of seeded grasses exhibited a significant difference among fertilization levels in 1979 and 1981 (Fig. 10). Both levels of fertilizer produced higher grass biomass than was produced on control plots that were unfertilized.

Forb production showed no significant difference among the three fertilizer levels in 1979 and 1981 (Fig. 10). Seeded shrubs showed a significant biomass response to fertilizer levels in 1979, but the response was not evident in 1981 because of the lower biomass of fourwing saltbush during the 1981 growing season (Fig. 10).

There was a general decrease of seeded grass and shrub biomass in 1981 compared to 1979 on all plots receiving fertilizer as well as the control plots (Fig. 10). This decline in production was probably due to low soil moisture in 1981 (15% by volume) compared to more favorable soil moisture (26% by volume) for plant growth in 1979. The decrease in yield from 1979 to 1981 was somewhat greater for the fertilized plots than the control plots; however, the differences were not significant in all cases.

Invasion

Biomass of invading grasses was significantly affected by seed mixture in 1979 and 1981 (Fig. 8). In 1979, biomass of invading grasses was greatest in the native and combination grass-forb-shrub mixture, intermediate in the native grass and native grass-forb mixtures, and lowest in the introduced grass and introduced grass-forb mixtures. In 1981, invading grass biomass was greatest on the native grass and native grass-forb-shrub mixtures, intermediate in the combination grass-forb-shrub and native grass-forb mixtures, and

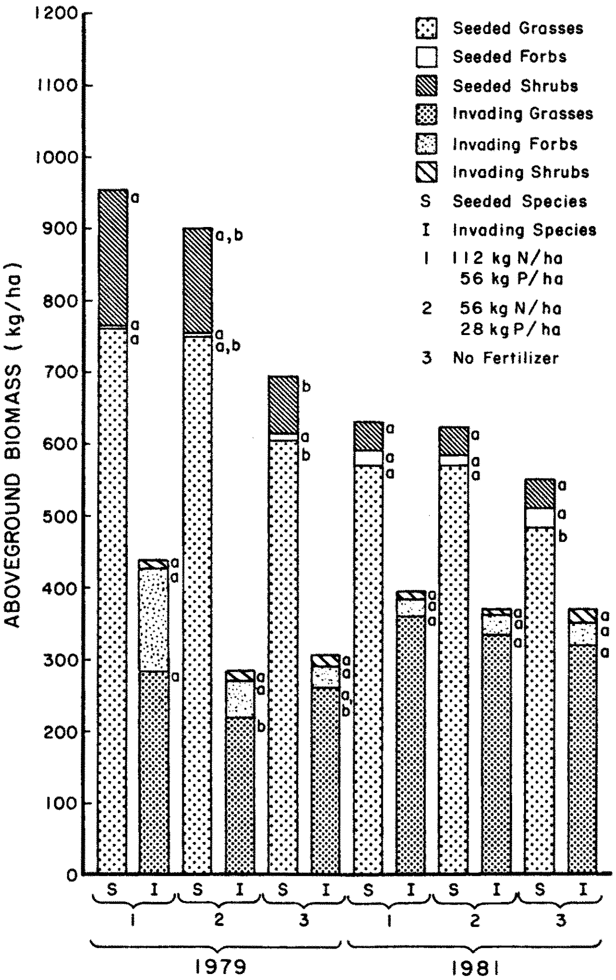

Fig. 10. Mean dry weight aboveground biomass of invading and seeded grasses, forbs, and shrubs by fertilizer treatment in 1979 and 1981. Means with different letters within life forms are significantly different (p=0.10).

lowest in the introduced grass and introduced grass-forb mixtures (Fig. 9). Generally, there was less grass invasion in introduced mixtures compared to native mixtures. This may be partially caused by increased root competition from introduced species compared to native species and also by timing of growth initiation which was somewhat earlier for the introduced grasses compared to most of the native grasses.

Cheatgrass (Bromus tectorum) is the dominant invading grass. It begins growth in late February and grows through the middle of May (Hull 1949). Crested wheatgrass generally begins growth before or at the same time as cheatgrass and therefore may survive the competition , but other later growing grasses may find that cheatgrass has used all or most of the available surface moisture before they initiate spring growth. Therefore, established crested wheatgrass plants may be effectively competing with cheatgrass for moisture at the critical seedling emergence stage. The increase in cheatgrass production of 140 kg/ha between 1979 and 1981 in the native grass mixture may be attributed to the decline in competition from invading forbs which declined 230 kg/ha during the same time (Figs. 8 and 9).

Biomass of invading forbs was significantly greater in the native grass and native grass-forb-shrub mixtures compared to all other mixtures in 1979 (Fig. 8). Similarly, forb invasion was greater (although not usually significant) in native mixtures compared to introduced mixtures in 1981. This may be caused in part by faster and greater occupancy of available space by introduced grasses compared to native species. The continuation of this trend may be caused by scarlet globemallow (Sphaeralcea coccinea). The rhizomatous nature of this perennial invading forb species enables it to quickly populate open areas and be a permanent component of plant communities.

Biomass of invading shrubs was less than 35 kg/ha and was not significantly different between seed mixtures in 1979 and 1981 (Figs. 8 and 9). Although seed source is present for big sagebrush, rabbitbrush, and broom snakeweed, the opportunity for shrub seedlings to successfully establish under intense competition with the seeded species appears to be highly limited.

Invading grass production was greater under high fertilization (112 kg N/ha and 56 kg P/ha) compared with the low fertilization level and no fertilization in 1979 (Fig. 10). Invading grass production was not significantly different between fertilizer treatments in 1981. The generally low response by invading grasses to fertilization in 1979 and 1981 may have been caused by competition from seeded species for moisture, space, and light.

Invading forbs and shrubs exhibited no significant differences to fertilizer response for the growing seasons of 1979 or 1981. Invading forb and shrub production was generally low except for forb production on the high fertilizer rate in 1979. Therefore, other factors including available seed source, moisture, and unoccupied space probably are more critical to the establishment and production of invading forbs and shrubs than are soil fertility levels.

Conclusions

All seed mixtures tested have proven to be tolerant to short-term droughts after establishment. Both introduced and native species mixtures had generally similar total production in 1979 and 1981. Therefore, any of the tested mixtures could be selected according to post-mined land use because the mixtures have demonstrated short-term adaptability and survival.

Although introduced mixtures were slightly more resistant to invasion compared to native mixtures, seeded species production in native mixtures was not sufficiently lower compared to introduced mixtues to preclude their use in areas of potentially high invasion.

Fertilizer was effective in increasing seeded grass and shrub production and cover in 1979 and seeded grass production and cover in 1981. Therefore, if grass production and cover needs to be increased rapidly, then fertilization is an efficient method without increasing production of undesirable invading species.

Introduced grasses (Russian wildrye, crested wheatgrass, and pubescent wheatgrass) and native grasses (beardless bluebunch wheatgrass and streambank and thickspike wheatgrass) were adapted and aggressive species on this site. However, some grass species including Indian ricegrass, green needlegrass, and meadow brome did not contribute a substantial percentage to the species composition. Similarly, most forbs found to be adapted to this site had low densities and production, except for alfalfa. Therefore, a reduction in the seeding rate of aggressive grasses and an increase in the seeding rate of less aggressive grass and forb species may be an alternative to altering species composition if more diversity is important to the post-mined land use.

Invading species can compose a substantial percent of the biomass in seeded stands. Annual species including cheatgrass and chenopods may not be desirable species in the plant community. They are dependent on moisture for production each year and, therefore, in dry years production would be low and little growth would be available for livestock and wildlife foods and for erosion control. Similarly, their annual growth habit indicates that potential erosion problems would be present during the season when cheatgrass and chenopods are not growing. Also, these species compete with perennial species that are more suitable (due to more stable production levels) for erosion control and as food for livestock and wildlife. Other invading species such as globemallow, rabbitbrush, and sagebrush may contribute to the stability and diversity of a reseeded community. They may increase the use of limited resources by using portions of the resources that were not being used by other species. Deep-rooted shrubs can recover and use deep soil moisture and nutrients that are below grass rooting depths. Also, palatable plant parts can increase the animal carrying capacity of the land or lengthen the grazing season. Because invading species may have different requirements for germination and survival, they may offer an inexpensive way to establish vegetation on small, bare areas that may not be easily vegetated by the seeded species.

High and Low Elevation Revegetation Study (Satellite Areas)

Introduction

State and federal reclamation laws require that life form diversity in seeded plant communities be approximately equal to that in the native community. This goal is often difficult to achieve because seeds differ in germination rates and responses to environmental conditions. Therefore, mixtures containing a certain proportion of these seeds will not always result in the same proportion when the stand becomes established. Previous studies have shown that sites favorable to grass growth may require twice as much shrub seed as grass seed to obtain even one-fourth as much shrub biomass (Redente and Cook 1981). Conversely, on rocky soils or other sites unfavorable to grasses, seeding rates of grasses must be increased to ensure that grasses comprise a substantial portion of the resulting stand. Factors controlling seed mixture expression are not understood well enough to accurately predict what proportion of grasses, forbs, and shrubs will occur in a stand following the use of a given seed mixture. The Satellite Study Areas were initiated in fall of 1980 to determine the effects of altering forb-grass and shrub-grass seed ratios on establishment and composition of life forms on disturbed sites at high and low elevations.

Site Description

The low elevation site is located at 1,988 m in a big sagebrush-grassland community. The native vegetation is dominated by big sagebrush with Douglas rabbitbrush (Chrysothamnus viscidiflorus) and fringed sage (Artemisia frigida) as common shrub components. Scarlet globemallow, wild buckwheat (Eriogonum spp.), nodding onion (Allium cernuum), phlox (Phlox spp.), Indian ricegrass, needleandthread, western wheatgrass , prairie junegrass, squirreltail (Sitanion hystrix), and cheatgrass occur throughout the understory. The average annual precipitation for this site varies from 33-38 cm with slightly less than one-half occurring as snow. The soil at the low elevation plot is a shallow (32-60 cm deep), calcareous (pH 8.4), gravelly fine sandy loam formed from sandstone. It is classified as a sandy skeletal, mixed, mesic, ustic torriorthent.

The high elevation plot is located at 2,440 m in a mixed mountain shrub community. The native vegetation is composed primarily of serviceberry (Amelanchier spp.), antelope bitterbrush, mountain mahogany (Cercocarpus montanus), and Gambels oak (Quercus gambelii). Other less dominant species include western wheatgrass, prairie junegrass, sheep fescue (Festuca ovina), mountain brome (Bromus carinatus), lupine (Lupinus spp.), wild buckwheat, arrowleaf balsamroot, phlox, and Douglas rabbitbrush. The site receives 45-55 cm of annual precipitation with approximately 65% occurring as snow. The soil at the high elevation site is a shallow (12-22 cm deep), noncalcareous (pH 7.2), fine sandy loam formed from sandstone. It is classified as a loamy, skeletal, mixed, lithic cryoboroll.

Site Preparation

In July of 1980 the vegetation and approximately 5-10 cm of soil were scraped from the sites. The seedbeds were disced to a depth of 10 cm. Each site was divided into sixteen 3x6 m subplots arranged in a randomized block design. The plots were broadcast seeded by hand and raked to cover the seed with soil.

Tables 5 and 6 show the four seed mixtures used on the two sites. The seeding rates of forbs and shrubs were equal to that of grasses in Seed Mixtures 1 and 3, whereas in Seed Mixtures 2 and 4 the seeding rates of forbs and shrubs were doubled and the seeding rate of grasses was reduced by one-half.

Table 5. Seed mixtures for the High Elevation Revegetation Plot.

Species	Mixture 1 PLS†/ft^2	Mixture 1 Kg PLS/ha	Mixture 2 PLS/ft^2	Mixture 2 Kg PLS/ha	Mixture 3 PLS/ft^2	Mixture 3 Kg PLS/ha	Mixture 4 PLS/ft^2	Mixture 4 Kg PLS/ha
Grass-forb								
Western wheatgrass	4.0	5.8	2.0	2.9				
Sodar streambank wheatgrass	4.0	1.4	2.0	0.7				
Shermans big bluegrass	3.0	0.2	1.5	0.1				
Durar hard fescue	3.0	0.3	1.5	0.2				
Slender wheatgrass	3.0	1.1	1.5	0.6				
Thickspike wheatgrass	3.0	1.2	1.5	0.6				
Sweetvetch	7.0	10.8	14.0	21.7				
Lutana cicer milkvetch	6.0	2.2	12.0	4.4				
Globemallow	7.0	0.6	14.0	1.3				
Total	40.0	23.6	50.0	32.5				
Grass-shrub								
Western wheatgrass					4.0	5.8	2.0	2.9
Sodar streambank wheatgrass					4.0	1.4	2.0	0.7
Shermans big bluegrass					3.0	0.2	1.5	0.1
Durar hard fescue					3.0	0.3	1.5	0.2
Slender wheatgrass					3.0	1.1	1.5	0.6
Thickspike wheatgrass					3.0	1.2	1.5	0.6
Bitterbrush					3.0	12.6	6.0	25.3
Winterfat					3.0	5.3	6.0	10.6
Serviceberry					3.0	6.5	6.0	13.1
Mountain mahogany					4.0	6.1	8.0	12.2
Golden currant					5.0	1.4	10.0	2.8
Skunkbush					3.0	8.8	6.0	17.5
Total					41.0	50.7	52.0	86.6

†Pure live seed.

Table 6. Seed mixtures for the Low Elevation Revegetation Plot.

Species	Mixture 1 PLS†/ft^2	Mixture 1 Kg PLS/ha	Mixture 2 PLS/ft^2	Mixture 2 Kg PLS/ha	Mixture 3 PLS/ft^2	Mixture 3 Kg PLS/ha	Mixture 4 PLS/ft^2	Mixture 4 Kg PLS/ha
Grass-forb								
Indian ricegrass	3.0	0.9	1.5	0.5				
Bearded bluebunch wheatgrass	3.0	1.2	1.5	0.6				
Western wheatgrass	4.0	6.0	2.0	3.0				
Green needlegrass	3.0	1.2	1.5	0.6				
Sodar streambank wheatgrass	4.0	1.4	2.0	0.7				
Thickspike wheatgrass	3.0	1.2	1.5	0.6				
Sweetvetch	8.0	17.4	16.0	34.7				
Lutana cicer milkvetch	7.0	3.9	14.0	7.8				
Globemallow	5.0	0.6	10.0	1.3				
Total	40.0	33.8	50.0	49.8				
Grass-shrub								
Indian ricegrasss					3.0	0.9	1.5	0.5
Bearded bluebunch wheatgrass					3.0	1.2	1.5	0.6
Western wheatgrass					4.0	6.0	2.0	3.0
Green needlegrass					3.0	1.2	1.5	0.6
Sodar streambank wheatgrass					4.0	1.4	2.0	0.7
Thickspike wheatgrass					3.0	1.2	1.5	0.6
Fourwing saltbush					4.0	13.8	8.0	27.5
Winterfat					4.0	6.8	8.0	13.6
Green ephedra					3.0	7.4	6.0	14.7
Shadscale					3.0	4.2	6.0	8.3
Big sagebrush					3.0	0.1	6.0	0.3
Douglas rabbitbrush					3.0	0.5	6.0	0.9
Total					40.0	44.7	50.0	71.3

†Pure live seed.

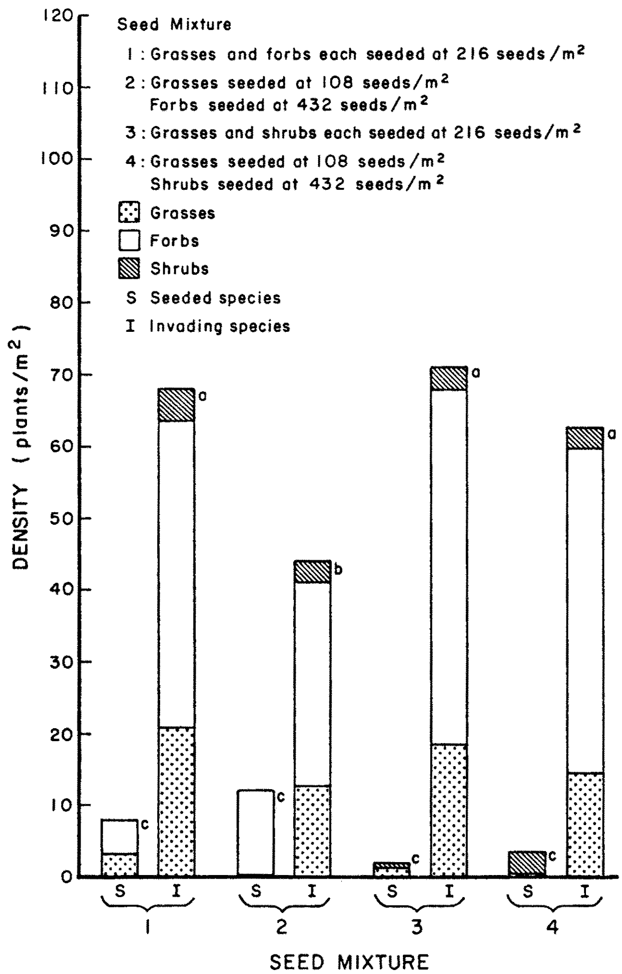

Fig. 11. Density of seeded and invading grasses, forbs, and shrubs for each seed mixture on the Low Elevation Revegetation Study after one growing season (1981). Columns with the same letter are not significantly different (p=0.10).

Ten permanently marked 0.25 m^2 quadrats were placed in each subplot. At the end of the first growing season density, aboveground biomass, and percent canopy cover were estimated for all invading and seeded plant species using nondestructive techniques. Analysis of variance and Tukey's test were used to determine significant differences among the means of each subplot.

Results and Discussion

Seeds sometimes remain in the soil for one or more years before germination occurs. This delay may be caused by seed dormancy or improper environmental conditions such as temperature and moisture. Since the results reported in this study are based on data collected after one growing season, information will only be available on species whose germination requirements have been met during the first growing season. Therefore, all aspects of seed mixture expression will not be apparent for at least another year. Nevertheless, an analysis of the initial stages of seedling establishment can be useful for understanding the mechanisms involved in plant community establishment.

Low Elevation Site

Seeded vs Invading Species

After one growing season, establishment of seeded species was low (Fig. 11). Only 0.6% of the grass seeds, 2.8% of the forb seeds, and 0.6% of the shrub seeds produced grass, forb, or shrub seedlings.

Invading species comprised 78-97% of the plant density, 97% of the aboveground biomass, and 96% of the canopy cover over the plots. Meteorological records show that precipitation during the winter and spring months following seeding was approximately 40% below normal. This may be the primary reason that seeded species did so poorly and invading species predominated. Invaders commonly have two strategies that allow them to be less affected by low rainfall than are seeded species. First, they are often able to germinate and carry out root growth during periods of low temperature (Harris 1967). This allows them to take advantage of the moisture available early in the growing season. Second, invaders tend to be xerophytic (Daubenmire 1968) and are thereby able to persist for longer time periods when water is deficient. Therefore, the low moisture present at the low elevation site probably inhibited germination and establishment of seeded species more so than invading species.

The primary invaders were lambsquarter (*Chenopodium album*), amaranth (*Amaranthus hybridus*), scarlet globemallow, longleaf phlox (*Phlox longifolia*), and wild buckwheat (Table 7). Density, biomass, and cover of the invading species were similar for all seed treatments except Seed Mixture 2 (Fig. 11). There was lower establishment of invaders in Seed Mixture 2. This mixture also had the highest establishment rate for seeded species (primarily forbs). Increased amounts of seeded forbs may have provided additional competition which reduced establishment of invading species on these plots.

Effects of Altering Seed Ratios

Doubling the seeding rates of forbs resulted in twice as much forb establishment compared with forbs seeded at the same rate as grasses (Fig. 12). Approximately 75% of the forb seedlings were sweetvetch and the rest were cicer milkvetch. Globemallow failed to become established during the first growing season on any of the plots.

Doubling the seeding rate of shrubs significantly increased seeded shrub density (Fig. 12). Seeding shrubs at 32.7 kg/ha resulted in an average density of 0.75 plants/m^2 while a rate of 64.4 kg/ha resulted in an average density of 3 plants/m^2. Biomass and cover values also increased, but not significantly. Shadscale (*Atriplex confertifolia*), fourwing saltbush, and

Table 7. Density, aboveground dry weight biomass, and canopy cover of invading grasses, forbs, and shrubs on Low Elevation Plots after one growing season (1981). Values were averaged over all seed mixtures.

Common Name	Scientific Name		Density (plants/m²)	Aboveground Biomass (kg/ha)	Canopy Cover (%)
Grasses					
Wheatgrass	*Agropyron* spp.		4.2	3.0	0.2
Russian wildrye†	*Elymus junceus*		1.0	2.1	0.1
Cheatgrass	*Bromus tectorum*		0.1	0.3	0.1
Others			12.0	2.4	0.3
		Subtotal	17.3	7.8	0.7
Forbs					
Lambsquarter	*Chenopodium album*		2.2	74.7	2.1
Scarlet globemallow	*Sphaeralcea coccinea*		18.0	37.4	3.7
Amaranth	*Amaranthus hybridus*		0.8	25.3	0.4
Longleaf phlox	*Phlox longifolia*		16.8	9.5	0.8
Wild buckwheat	*Eriogonum* spp.		0.7	10.7	0.4
Others			3.1	7.2	0.4
		Subtotal	41.6	164.8	7.8
Shrubs‡					
Gardner saltbush	*Atriplex gardneri*				
Douglas rabitbrush	*Chrysothamnus viscidiflorus*				
Green ephedra	*Ephedra viridis*				
		Subtotal	2.7	3.5	0.2
Total			61.6	176.1	8.7

†Volunteer plants from previous study.

‡These species not measured individually.

Table 8. Density, aboveground dry weight biomass, and canopy cover of invading grasses, forbs, and shrubs on High Elevation Plots after one growing season (1981). Values were averaged over all seed mixtures.

Common Name	Scientific Name		Density (plants/m²)	Aboveground Biomass (kg/ha)	Canopy Cover (%)
Grasses					
Brome grasses	*Bromus* spp.		8.5	1.0	1.1
Wheatgrasses	*Agropyron* spp.		3.8	0.4	0.4
Others			8.4	0.5	0.5
		Subtotal	20.7	1.9	2.0
Forbs					
Lupine	*Lupinus argentea*		6.7	7.0	4.5
Astragalus	*Astragalus tenellus*		3.3	0.3	0.3
Lambsquarter	*Chenopodium album*		0.2	0.6	0.1
Chenopodium	*Chenopodium* spp.		0.2	0.5	0.3
Polygonum	*Polygonum* spp.		1.3	0.3	0.3
Others			13.3	1.0	1.0
		Subtotal	25.0	9.7	6.5
Shrubs‡					
Douglas rabbitbrush	*Chrysothamnus vicidiflorus*				
Serviceberry	*Amelanchier alnifolia*				
Big sagebrush	*Artemisia tridentata*				
		Subtotal	1.3	1.0	0.6
Total			47.0	12.6	9.1

‡These species not measured individually.

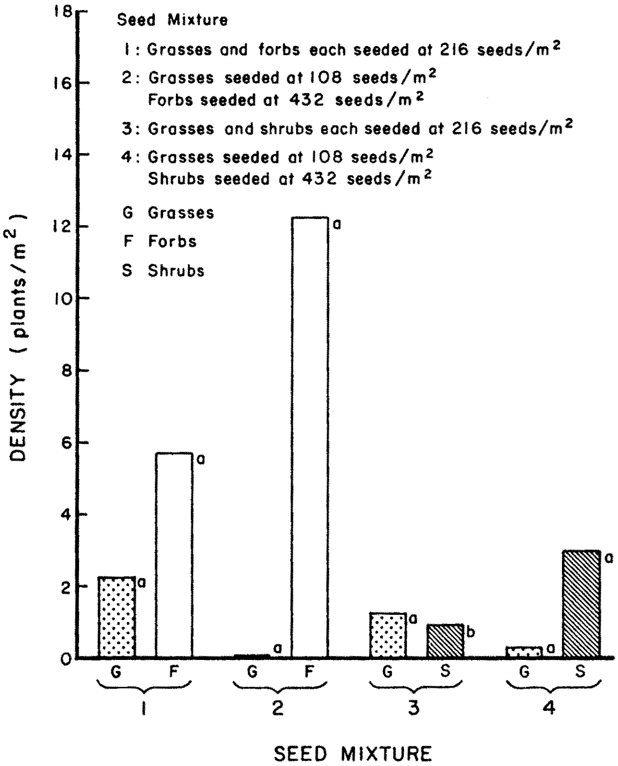

Fig. 12. Density of seeded grasses, forbs, and shrubs for each seed mixture on the Low Elevation Revegetation Study after one growing season (1981). Columns with the same letter in each life form are not significantly different (P=0.10).

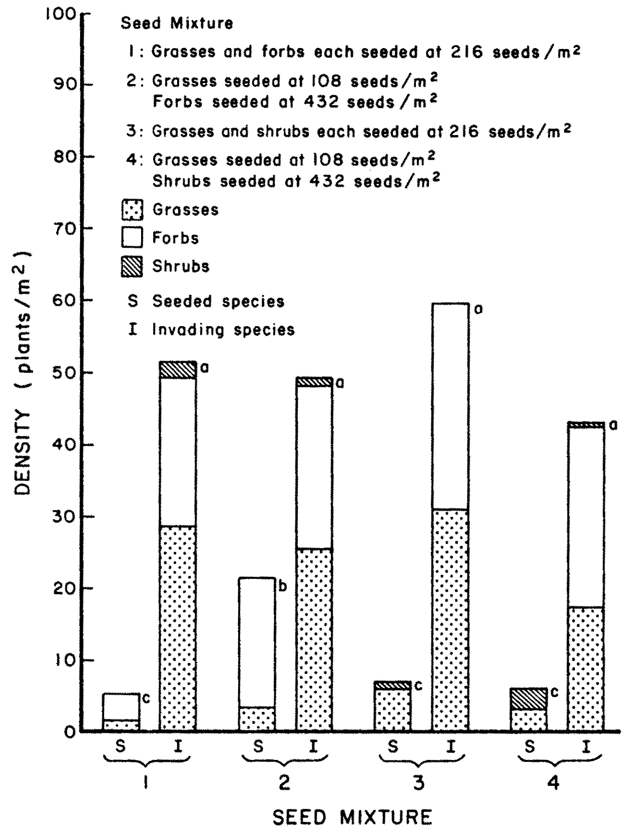

Fig. 13. Density of seeded and invading grasses, forbs, and shrubs for each seed mixture on the High Elevation Revegetation Study after one growing season (1981). Columns with the same letters in each life form are not significantly different (P=0.10).

green ephedra were the only seeded shrubs to become established.

Reducing the seeding rate of grasses by one-half resulted in low grass densities. Densities dropped from 2.3 plants/m^2 to almost zero in the grass-forb mixture and from 1.3 plants/ m^2 to 0.3 plants/m^2 in the grass-shrub mixture (Fig. 12). Indian ricegrass, western wheatgrass, green needlegrass, and streambank wheatgrass were the principle seeded grasses to become established.

High Elevation Site

Seeded vs Invading Species

Invading species dominated the stands on the high elevation site (Fig. 13). Invaders comprised approximately 84% of the plant density, 94% of the aboveground biomass, and 93% of the canopy cover. Establishment rates of seeded grasses, forbs, and shrubs were considered low. Only 2.1% of the grass seeds, 2.9% of the forb seeds, and 0.6% of the shrub seeds produced plants the first growing season. As in the low elevation site, low rainfall during the winter and spring months probably inhibited germination and establishment of the seeded species.

At the high elevation site grasses and forbs comprised nearly equal proportions of the invading species. Invading grasses included bromegrasses, wheatgrasses, Indian ricegrass, and needleandthread (Table 8). The primary invading forbs were lupine (Lupinus argentea), astragalus (Astragalus tenellus), and polygonum (Polygonum spp.). Invading shrubs such as Douglas rabbitbrush, serviceberry, and big sagebrush comprised a minor portion of the total stand.

Effects of Altering Seed Ratios

Doubling forb seeding rates and reducing grass seeding rates by one-half significantly increased the density, biomass, and cover of seeded forbs (Fig. 14). Approximately two-thirds of the forb seedlings were sweetvetch; the remainder were cicer milkvetch. Globemallow did not show any emergence in any of the plots.

Density, biomass, and cover of seeded shrubs also increased when shrub seeding rates were doubled and grass seeding rates were halved. Golden

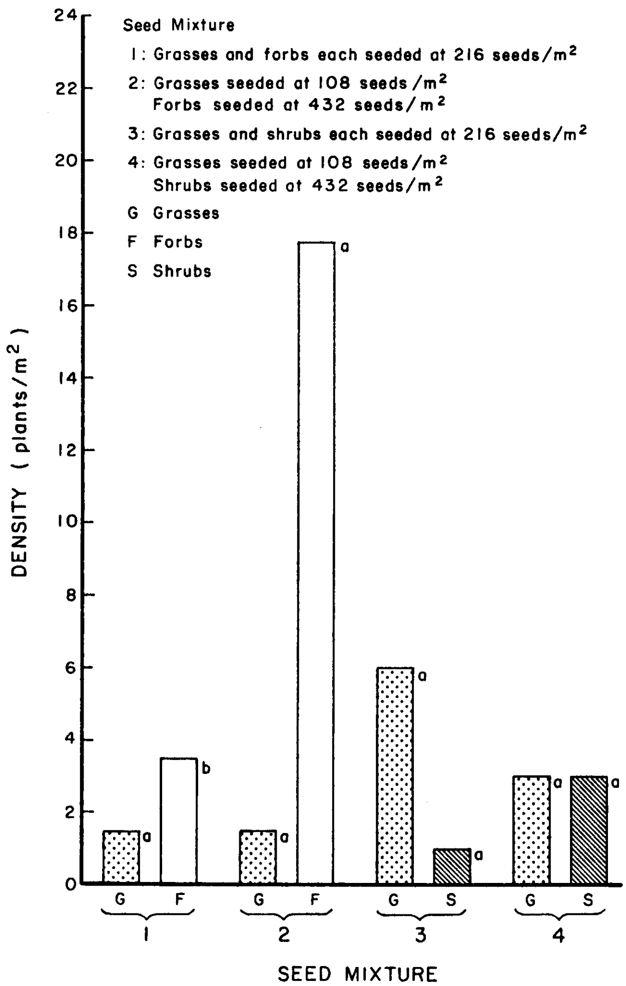

Fig. 14. Density of seeded grasses, forbs, and shrubs for each seed mixture on the High Elevation Revegetation Study after one growing season (1981). Columns with the same letter in each life form are not significantly different (P=0.10).

current (Ribes aureum), skunkbush (Rhus trilobata), and serviceberry (Amelanchier alnifolia) were the primary seeded shrubs to establish during the first growing season.

Densities of seeded grasses were similar for all seed mixtures. Apparently, reducing grass seeding rates by one-half had little effect on the establishment of seeded grasses. Bearded bluebunch wheatgrass (Agropyron spicatum) and western wheatgrass were the only seeded grasses to become established at either seeding rate.

Conclusions

1. At both high and low elevation sites, seeded species established at low rates. Invading species dominated the stands. This was most likely a result of low rainfall during the winter and spring months which inhibited growth of seeded species more than invading species.

2. At the high elevation site, where conditions were more favorable to grasses, low seeding rates (5.1 kg/ha) could be used without reducing early establishment. At the low elevation site, however, higher rates (11.9 kg/ha) should be used in order to obtain successful establishment of grass species.

3. Doubling the seeding rates of forbs and shrubs and reducing seeding rates of grasses in the mixture doubled the rate of early establishment of forbs and shrubs at both sites.

Seeding Depth Study

Introduction

Fifteen species of forbs, grasses, and shrubs (Table 9) were planted in greenhouse flats at four depths (0.6, 1.3, 1.9, and 2.5 cm below the soil surface) in a clay soil and in a sandy loam soil to examine the effects of seed depth and soil texture on emergence success. Species were selected because of their potential importance for revegetation but knowledge concerning appropriate seeding depth for them was lacking. Clay and sandy loam soils were selected because these textures are found to be major types in the Piceance Basin overlying the rich oil shale deposits in Colorado.

A split plot randomized block design was used under controlled greenhouse conditions. Each of four blocks had all species planted at all depths.

Table 9. Species seed in the Seeding Depth Study.

Common Name	Scientific Name
Grasses	
Sand dropseed†	Sporobolus cryptandrus
Alkali sacaton	S. airoides
Green needlegrass	Stipa viridula
Western wheatgrass	Agropyron smithii
Indian ricegrass	Oryzopsis hymenoides
Durar hard fescue	Festuca ovina duriuscula
Shermans big bluegrass	Poa ampla
Forbs	
Lewis flax†	Linum lewisii
Scarlet globemallow†	Sphaeralcea coccinea
Palmer penstemon†	Penstemon palmeri
Shrubs	
Fourwing saltbush	Atriplex canescens
Green ephedra	Ephedra viridis
Antelope bitterbrush†	Purshia tridentata
Big sagebrush†	Artemisia tridentata
Mountain mahogany†	Cercocarpus montanus

†Species with low emergence that were not reported in the results.

Each species was planted in 60-cm-long rows with 25 seeds planted per species. Soil moisture was maintained at field capacity during the study. Emergence was monitored daily for 30 days. The number of emerged plants was recorded daily by species, depth, and block. Data were analyzed and statistical significance determined at the p=0.10 level using Friedman's two-way analysis of variance. Of the fifteen species planted, only the results of eight species are reported. The results of the other seven species are not reported because of their low emergence (Table 9).

Results and Discussion

Effect of Soil Texture on Seedling Emergence

Soil types significantly affected emergence of five species. Fourwing saltbush, big bluegrass, green needlegrass, alkali sacaton (*Sporobolus airoides*), and Indian ricegrass had significantly greater emergence in sandy loam soils compared to clay soils (Table 10). In addition, hard fescue (*Festuca ovina duriuscula*) had greater emergence in sandy loam soil compared to clay soil, although the difference was not significant. Fourwing saltbush (Stark 1966), big bluegrass, green needlegrass (Heady 1975), alkali sacaton (Sampson 1924), Indian ricegrass (Vallentine 1971; Montana Agricultural Experiment Station 1970), and hard fescue (U.S. Forest Service 1937) have been reported to have equal or better emergence potential on coarse and medium soils compared to fine textured clays although some of these species including alkali sacaton (Vallentine 1971; Hafenrichter et al. 1968), big bluegrass (Vallentine 1971), and green needlegrass (Atkins and Smith 1967) have also been shown to perform better on clays compared to lighter soil textures. Green ephedra had greater emergence on clay soil compared to sandy loam soil, although the differences were not significant. Stark (1966) indicated that green ephedra generally did better on medium to coarse textured soils. Western wheatgrass had about equal emergence on clay and sandy loam soils as expected

Table 10. Mean percent emergence for all depths in clay and sandy loam soils.†

Species	Clay‡	Sandy‡ Loam
Fourwing saltbush	16.0b	25.0a
Green ephedra	35.3a	16.1a
Western wheatgrass	93.3a	93.8a
Shermans big bluegrass	24.7b	32.6a
Green needlegrass	53.7b	87.0a
Durar hard fescue	43.3a	56.3a
Alkali sacaton	2.9b	59.1a
Indian ricegrass	10.0b	69.5a

†Percent emergence was adjusted for live seed.

‡Numbers with different letters in a row are significantly different (p=0.10).

because of its adaptability to many soil textures (Stefferud 1961; Allred and Nixon 1955). Most of these previous findings were based on field studies or on general ecological observations. Soil moisture, temperature, and competition were not controlled in many instances. These factors may modify outcomes of emergence; therefore, some differences should be expected between a controlled greenhouse experiment and less controlled field studies.

Effect of Planting Depth on Seedling Emergence

Planting depth significantly affected emergence of seven of the 15 species planted (Table 11). Generally emergence was greatest at 0.6-cm depth, lowest at 2.5-cm depth, and intermediate at 1.3-cm and 1.9-cm depths. This trend was not exhibited by western wheatgrass, green ephedra, and green needlegrass emergence in sandy loam soil and Indian ricegrass emergence in both sandy loam and clay soils. Western wheatgrass (Stefferud 1961; Archer and Bunch 1953; Allred and Nixon 1955), green ephedra (Williams et al. 1974; Kay et al. 1977), green needlegrass (McWilliams 1955), and particularly Indian ricegrass (Hafenrichter et al. 1968) have recommended planting depths of 1.3 cm or greater.

Generally, the greatest variation between planting depths was on clay soils. This may have been caused by the physical characteristics of heavy clays. Fourwing saltbush, Indian ricegrass, and alkali sacaton did not have greater variation of emergence between planting depths in clay soil compared to sandy loam soil (Table 11). The low emergence variation between depths in clay may have

Table 11. Mean percent emergence at four planting depths in sandy loam and clay soils.†

Species	Soil Texture‡	Planting Depth§ 0.6 cm	1.3 cm	1.9 cm	2.5 cm
Green ephedra	C	57.7a	37.8b	35.6b	11.1c
	L	21.1a	8.9a	18.9a	15.6a
Fourwing saltbush	C	16.3a	15.1a	19.8a	12.8a
	L	37.2a	29.1a	16.3b	17.4b
Western wheatgrass	C	104.5a	98.90ab	83.1c	86.5bc
	L	95.5a	95.5a	94.4a	89.9a
Big bluegrass	C	64.4a	17.8b	9.6c	6.8d
	L	52.1a	38.4a	28.8b	11.0c
Green needlegrass	C	91.8a	60.3ab	54.8b	8.2c
	L	83.6a	95.9a	86.3a	82.2a
Hard fescue	C	100.0a	39.ab	16.3c	17.4bc
	L	93.5a	62.0ab	48.9ab	20.7b
Alkali sacaton	C	5.8a	4.7ab	1.2ab	0.0b
	L	62.8ab	70.9a	51.2b	51.2b
Indian ricegrass	C	5.0a	17.5a	2.5a	15.0a
	L	65.0a	70.0a	60.0a	82.5a

†Percent emergence was adjusted for live seed and sometimes exceeds 100% because of random variation.

‡C = clay soil texture; L = sandy loam soil texture.

§Numbers with different letters within a row are significantly different (p=0.10).

been caused by the generally low number of emerging alkali sacaton, Indian ricegrass, and fourwing saltbush plants rather than a true indication of the effect of planting depth in clay.

Conclusions

The data presented indicates that coarser textured soils generally allow better overall plant emergence compared to finer textured soils. Therefore, topsoiling with loam and sandy loams (or overlaying clay with these soil textures) may provide greater species performance compared to clays. Also, the reduced effect of planting depth in sandy loam compared to clay indicates that greater latitude can be used for selecting planting depths on reclamation sites with sandy loam soils. Seeding depth data can provide insights on how to adjust species composition in mixtures to increase the early composition of species that have poor emergence. However, these data must be interpreted in light of field use where moisture may be more limiting and variable at different depths to provide a more complete evaluation of potential species response on sites to be reclaimed.

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SOIL MICROORGANISMS AND MANAGEMENT OF RETORTED SHALE RECLAMATION

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OBJECTIVES

The effects of soil disturbance and retorted oil shale on the development of soil microbial processes have been monitored in relation to seeding mixture, fertilization, irrigation, and seeding variables. Specific objectives include the following:

1. To evaluate microbial responses during reestablishment of plant communities on disturbed and revegetated soils using plots established at the Intensive Study Site.
2. To study the effects of soil storage and disturbance on microbiological populations and on microorganism-related nutrient cycling processes.
3. To determine the effects of retorted shale on the microbiological characteristics and activities in surface soils placed over the retorted material as it relates to revegetation potential.
4. To study belowground responses using reclamation plots in western Colorado which have been monitored for plant development on retorted shale materials and which were constructed in 1971-1974.

METHODS

Study Site

Most of the sampling was completed at the Intensive Study Site during the summer of 1981. In addition, several industry-constructed reclamation plots were also studied under the direction of personnel from the Department of Range Science at Colorado State University.

Soil Sampling

Soil samples were taken from the 5-10 cm depth to assure that effects of shorter-term variations in temperature and moisture would be minimized and to sample from the zone where maximum plant root development and microorganism activity would occur. Also, no aboveground materials or soils directly touching plants were taken in order to maintain the experimental integrity of the plots.

The off-site undisturbed control plot was sampled on May 22 and June 2.

The surface zone sampling of the Retorted Shale Successional Plot was completed on June 2 and July 13, 1981. Soil samples at greater depths were also taken on the Retorted Shale Successional Plots. A single core (30 cm into shale) was taken from each plot seeded to the native seed mixture on June 3-4, 1981. Soil cores were sectioned into 15-cm segments for analysis. The industry-constructed reclamation plots were sampled on June 29 and 30.

Samples were sieved through a 2-mm mesh screen, mixed in a Patterson-Kelly twin shell dry blender for 20 minutes, returned to plastic bags and stored at 6°C until analysis. The soils were double-bagged with moist toweling to minimize soil water loss.

Analytical Procedures

The procedures for soil organic matter measurement, viable microbial enumeration, nitrogen fixation, phosphatase and dehydrogenase activities, and soil ATP have been described by Hersman and Klein (1979) and Sorenson et al. (1981). The dehydrogenase assay was carried out using glucose amendments by adding 0.5 ml of a 1% glucose solution in place of distilled water.

Denitrification

Denitrification potential was measured using the acetylene inhibition technique (Yoshinari et al. 1977). Ten grams of soil were weighed into a 70-ml serum bottle and saturated by adding 4.5 ml of a solution containing 10 g glucose and 2 g KNO_3/l. The serum bottle atmosphere was replaced with N_2 by flushing for 5 minutes. Using a hypodermic syringe 6 ml of the serum bottle head space was withdrawn and then replaced with 6 ml of acetylene to give 10% acetylene content. The sample was then incubated for approximately 24 hours (actual time recorded). After incubation the bottle head space was analyzed for N_2O using a Varian Model 3700 gas chromatograph and high temperature electron capture detection. The GC column was 2m x 2.16mm ID stainless steel packed with Porapak Q (Waters Associates, Milford, Mass.) and was operated at 50°C. The gas chromatograph was programmed to recondition the column at 150°C for 5 minutes after each injection. Without reconditioning, unidentified slow eluting compounds interfered with subsequent analyses. The carrier gas was USP grade N_2. Carrier flow rate was 30 ml/min. The electron capture detector was operated at 300°C. Atmospheric levels of N_2O (~0.5 ppm by volume) could be detected using this system. Calibration was done using a commercial standard (Scientific Gas Products, Denver, Colo.) containing 100 ppm N_2O in N_2.

Nitrification

The status of autotrophic nitrifying microbial populations was determined using measurements of the ammonium ion and nitrite oxidation rates. The analytical time frame was kept short enough to exclude the proliferation of the bacteria primarily responsible for this activity. In this way a measure of the preexisting enzymatic activity of these organisms was obtained (Belser and Mays 1980; E. L. Schmidt, personal communication 1980).

To measure initial potential ammonium ion oxidation activity 12 g of soil were weighed into a 250-ml Erlenmeyer flask. Fifty milliliters of a 0.5 mm ammonium phosphate-buffer solution (167 mg K_2HPO_4/l, 3 mg KH_2PO_4/l, 66 mg $(NH_4)_2SO_4$/l, pH ~8) was added. To each flask 0.5 ml of 1 M $NaClO_3$ was added to block nitrite oxidation. The flasks were capped with aluminum foil and placed on an orbital shaker at 200 rpm and 24±2°C. Over the next 24-30 hours at 8-14 hour intervals, 5-7 ml samples of the soil slurry were poured from each flask into a test tube, $Ca(OH)_2$ (~0.1 g) was added to coagulate suspended clay, and the samples were centrifuged at 600 x g for 10 minutes. Triplicate 2-ml supernatant aliquots were diluted to 50 ml and analyzed for nitrite, and a least squares linear regression line was calculated to fit the nitrite concentratin of the slurry over time.

The initial potential nitrite oxidation rate was measured in a similar manner with 6.9 mg of $NaNO_2$ (0.1 mM) substituted for the $(NH_4)_2SO_4$ in the buffer solution used for ammonium oxidation rate measurements. A 20% solution of nitrapyrine (2-chlor-6-(trichloromethyl)pyridine) was added to each flask to block the oxidation of indigenous ammonium to nitrite (Shattuck and Alexander 1963), and similar nitrite concentration measurements were completed.

Ammonia Volatilization

Ammonia volatilization potential was measured using a gas scrubbing train in which air, saturated with water, was drawn across moist unamended or fertilized soil and then scrubbed through two tandem 0.01 N sulfuric acid traps. The apparatus was similar to that used by Klubeck et al. (1978).

Soil samples from the Retorted Shale Successional Plots planted with the native seed mixture were composited, and 100-g portions were used to measure ammonia volatilization. The samples were saturated with deionized water or solutions of $(NH_4)_2SO_4$ containing 100 mg N or 375 mg N/l to give added ammonium nitrogen contents of 0, 2.5, and 84.4 µg/g, respectively. The saturated soil was then centrifuged to the moisture equivalent (1-cm thick soil cake centrifuged at 1000 x g for 30 minutes), about $3x10^4$ pascals (Brady 1974) or 22-23% moisture. Each treatment was replicated three times, and triplicate blank trains (no soil) were run. At 6-7 day intervals the acid trapping solutions were removed from the scrub trains and analyzed for ammonia. All second trap solutions were negative. Soil flasks in the train were weighed, and small amounts of deionized water were added if needed to compensate for evaporative water loss. Incubation was continued for 34 days at room temperature.

Chemical/Physical Analyses

Soluble nitrate-nitrogen or nitrite-nitrogen were determined in 1:4 (w/v) extracts using either water or 1N KCl. Potassium chloride extracts were used when ammonium was also to be determined. Nitrate analyses used the cadmium reduction technique of Strickland and Parsons (1968). Generally, 2 ml of extract were diluted to 100 ml for analysis. The nitrite produced from reduction of nitrate on the cadmium columns plus indigenous nitrite was determined colorimetrically using the single color reagent of the U.S. EPA (1974). Absorbance was read at 540 nm using a 1-cm light path on a B&L Spectronic 100. Soil nitrate was calculated from a standard curve after correction for nitrite.

Nitrite was determined from the same extracts used for nitrate. Two milliliters of extract were diluted to 50 ml in deionized water, and color was developed using the reagent of the U.S. EPA (1974). Absorbance was read and nitrite-nitrogen calculated as described above for nitrate.

Ammonium ion was extracted using 1:4 (w/v) slurries of soil in 1N KCl shaken at 200 rpm and 24°C for 15-30 minutes (Bremner 1965). Following centrifugation at 600 x g for 10 minutes, the supernatant was assayed for NH_4^+-N using an ion selective electrode (HNU System, Inc., Newton, Mass., Model ISE-10-10-00) and a millivolt meter. Minimum detectable concentration of NH_4^+-N for this instrument was about 0.5 μg N/ml of extract or about 2.5 μg/g of soil.

Conductivity and water soluble ions and compounds were determined in saturated paste extracts of soil and processed oil shale by the Colorado State University Soils Laboratory. Major cations were determined using plasma emission spectroscopy (Dahlquist and Knoll 1978). Chloride was determined by titration with mercuric nitrate (American Public Health Association 1981). Thiosulfate determination was done using ion exchange techniques by the Center for Environmental Sciences Analytical Laboratory, University of Colorado at Denver.

Direct Counts of Bacteria

Direct microscopic counts of bacteria were completed using fluorescein isothiocyanate (FITC) staining and epifluorescent microscopy. The method generally follows that of Babiuk and Paul (1970). One gram of soil was blended at high speed with 99 ml of pH 9.6 bicarbonate buffer (9.89 g Na_2CO_3 plus 18.49 g $NaHCO_3$/l) for 2 minutes. Two 10-μl portions of the blended suspension were pipetted onto each of two 1-cm^2 wells of somatic cell count slides (Bellco Glass, Inc., Vineland, N.J.), and the suspension was spread to cover the area of the well. The suspensions were dried at 60°C and then stained by flooding with FITC stain. The stain solution consisted of 0.5 ml of pH 9.6 bicarbonate buffer (see above), 2.25 ml of pH 7.1 phosphate buffer (121 mg KH_2PO_4/l plus 529 mg K_2HPO_4/l), 2.24 ml 0.85% (w/v) NaCl, and 2 mg FITC. Stain was made fresh each day, protected from light, and kept cold in an ice bath. After staining for 3 minutes the preparations were decolorized for 10 minutes in a bath of pH 9.6 bicarbonate buffer and then for 2 minutes in a 5% (w/v) sodium pyrophosphate ($Na_4P_2O_7$) solution. The slides were gently rinsed with dionized water and dried at 60°C. Fluorescent bacteria and actinomycetes were counted at 1000 x magnification with oil immersion and incident illumination from an HBO 200 W/4 super pressure mercury lamp on a Zeiss Universal microscope with a Type II FL vertical illuminator. A minimum of 200 cells were counted per well, and the count and number of fields counted were recorded. Knowing the area of microscope field, the number of bacteria per gram of soil was calculated.

RESULTS

Undisturbed Control Study

The results from the Undisturbed Control Study are summarized in Table 12. The zymogenous dehydrogenase activity in samples collected on May 22, 1981 was very low, and several samples showed no activity increase with glucose additions. This

Table 12. Results from the Undisturbed Control Study. Twenty samples were taken May 22, 1981 and 10 were taken June 2, 1981.

Parameter	Unit	May 22 Mean	May 22 95% CI	June 2 Mean	June 2 95% CI
Zymogenous dehydrogenase	μg form./g · 24 h	4.1	7.3 - 1.0	34.0	41.5 -26.5
Autochthonous dehydrogenase	μg form./g · 24 h	35.9	47.0 - 24.8	49.4	66.7 -32.1
Phosphatase	μg PNP/g · h	117.8	134.7 -100.8	65.9	82.7 -49.0
Acetylene reduction	n moles C_2H_4/g · h	0.17	0.34- 0.01		
ATP	ng/g	106.1	123.5 - 88.7		
FITC bacteria	x 10^8/g	6.7	8.3 - 5.0		
"Viable" bacteria	x 10^6/g	4.2	5.0 - 3.4		
"Viable" actinomycetes	x 10^6/g	1.6	2.0 - 1.3		
Fungal propagules	x 10^4/g	4.1	4.6 - 3.5		
Ammonium ion oxidation	μg N/g · h			0.21	0.27- 0.1
Nitrite oxidation	μg N/g · h			0.11	0.14- 0.0
% organic matter	% dry weight	1.22	1.35- 1.09		
Hydrogen ion activity	pH	8.1	8.3 - 8.0		
% moisture	% dry weight	12.56	13.44-11.68	12.32	13.49-11.1

result suggests a strong seasonal influence on zymogenous hydrogenase activity in native, undisturbed soils of the study area.

Dehydrogenase activity was highest at the June sampling with a value of 34 µg formazan per gram of soil in 24 hours. This value is above the 95% confidence range for the mean zymogenous dehydrogenase activity in samples taken from the undisturbed control area in May and suggests the strong influence of short-term abiotic changes in these measurements. In similar comparisons, phosphatase activity was significantly lower in June; this decrease occurred when the zymogenous dehydrogenase activity showed an increase with advancement of season.

The average autochthonous dehydrogenase activity of the undisturbed control area was not significantly different in samples collected May 22 or June 2, 1981, which suggested that this measurement may be less sensitive to changes in plant growth and abiotic conditions. With these types of seasonal variations it is essential that time be minimized as a variable in experimental designs for activity measurements, except on cross-year analyses which should be interpreted with caution. More intensive control area monitoring would appear to be warranted based on these results.

Annual Disturbance Study

Using the original plots installed in 1977, the Annual Disturbance Plots were sampled using the same procedures as described in earlier reports.

By 1981 the most extremely disturbed treatment (#4) still had not reached the microorganism activity in the root zone of the control soil (Fig. 15). The dehydrogenase activity, especially, showed a more distinct response to disturbance than was indicated by the surface soil organic matter content. For these plots the second treatment, which was only moderately disturbed, showed no differences in soil organic matter while the plots #3 and #4 (more extreme disturbances) showed expected decreases due to the greater exposure of belowground soil materials for both dehydrogenase and phosphatase activities. In contrast, the denitrification activity showed a markedly different trend, in that the more severe disturbance treatment (#4) showed activity essentially the same as the control soils.

These results, together with the mycorrhizal analyses (Reeves et al. 1981), indicated that with mild disturbance recovery of the soil biological activity can occur under natural revegetation conditions but with more extreme disturbance an extended period may be required to have reestablishment of above- and belowground soil processes.

Retorted Shale Successional Study

Dehydrogenase Activity

Autochthonous dehydrogenase activity in samples collected July 13, 1981 showed significant fertilization and topsoil depth interactions (Fig. 16). The largest response to fertilization occurred with

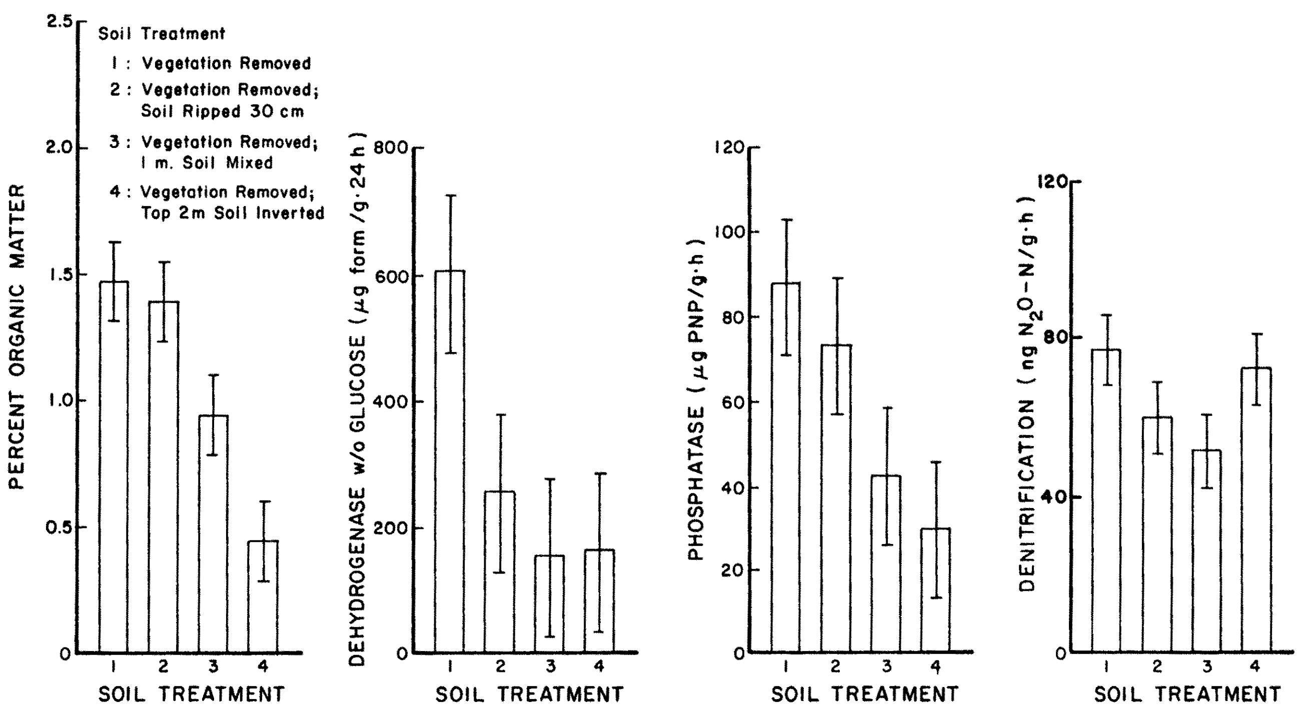

Fig. 15. Organic matter, dehydrogenase (control and with glucose), phosphatase, and denitrification potential for the Annual Disturbance Study--1981.

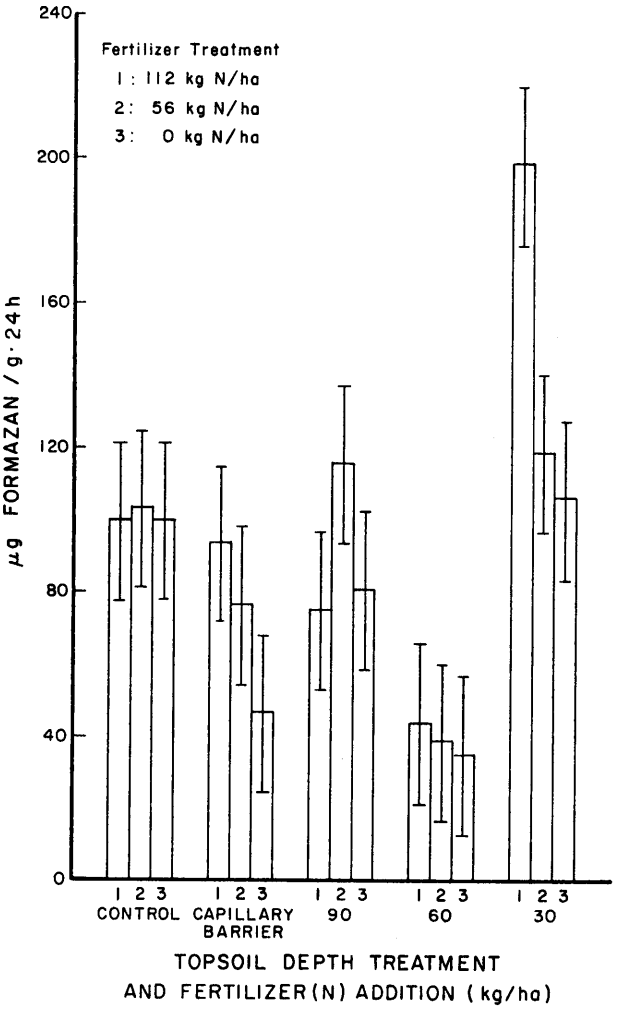

Fig. 16. Dehydrogenase activity without glucose amendments for the Retorted Shale Successional Study by soil depth treatment and fertilizer effects--July 1981.

with the higher fertilizer addition on soil 30 cm deep over shale. The only other statistically significant ($p \leq .05$) difference due to fertilization occurred with the capillary barrier between the retorted shale and the topsoil.

The average autochthonous dehydrogenase activity in any of the treatment plots (varying depths of topsoil over retorted shale) never exceeded the lower confidence interval for either the May or June samples from the undisturbed control area.

Zymogenous dehydrogenase responses in samples collected on June 23, 1981 were both higher and different from those observed at comparable dates in previous years. For the first time, activity in soil over the capillary barrier 90-cm deep over retorted shale was not significantly lower than that in the disturbed control soil; but, it was lower in the 60- and 30-cm deep soil placed over the retorted shale.

Zymogenous dehydrogenase activity in June showed a significant ($p \leq .05$) interaction between seed mixture (or the ensuing plant community), fertilization, and soil-shale arrangements. The nature of these interactions are illustrated in Fig. 17. This interaction was noted at both 1981 sampling dates and had not been detected previously.

The results of dehydrogenase assays for samples taken during the summers of 1979 through 1981 are shown in Figs. 18 and 19. In all of these figures results from the June 3, 1981 samples are used to compare with previous years. The autochthonous dehydrogenase activity in all soil-shale treatments for 1979, 1980, and June 1981 did not give similar or consistent responses over years with the treatments represented by the respective soil depths (Fig. 18). In addition, no fertilization treatments or seed mixture effects were evident.

The zymogenous dehydrogenase responses, in comparison, suggested that overall average activity had increased each year since 1979 (Fig. 19). In 1979 zymogenous dehydrogenase activity was very low in all of the soils directly over the processed shale. In addition, there was no significant difference between these treatments despite differences in soil depth over retorted shale. Zymogenous dehydrogenous activity was significantly higher in the soil over the capillary barrier than in those soils directly over shale, but activity in all of the replaced soils was lower than in the disturbed control soil.

The 1980 measurements indicated that there was no measurable increase in activity in the soil over the capillary barrier relative to that in the other replaced soils. However, activity in replaced soils remained significantly below that of the disturbed control soil.

Phosphatase Activity

In contrast to the zymogenous dehydrogenase activity, phosphatase activity has shown a consistent downward trend since 1979. Phosphatase activity in 30- and 60-cm deep soils over retorted shale has been consistently and significantly ($P \leq .05$) lower than in the disturbed control soil (Fig. 20).

When results from 1979, 1980, and June 2, 1981 are analyzed together a significant three-way interaction between fertilizer, seed mixture, and soil depth was observed (Fig. 21).

Nitrogen Cycling

Nitrogen Fixation Potential

Acetylene reduction activity has decreased in the treated plots during 1979, 1980, and 1981 (Fig. 22). For all three years acetylene reduction in the treatments of soil over retorted shale seemed to be severely reduced relative to the soils without underlying shale. In 1979 and 1981 this

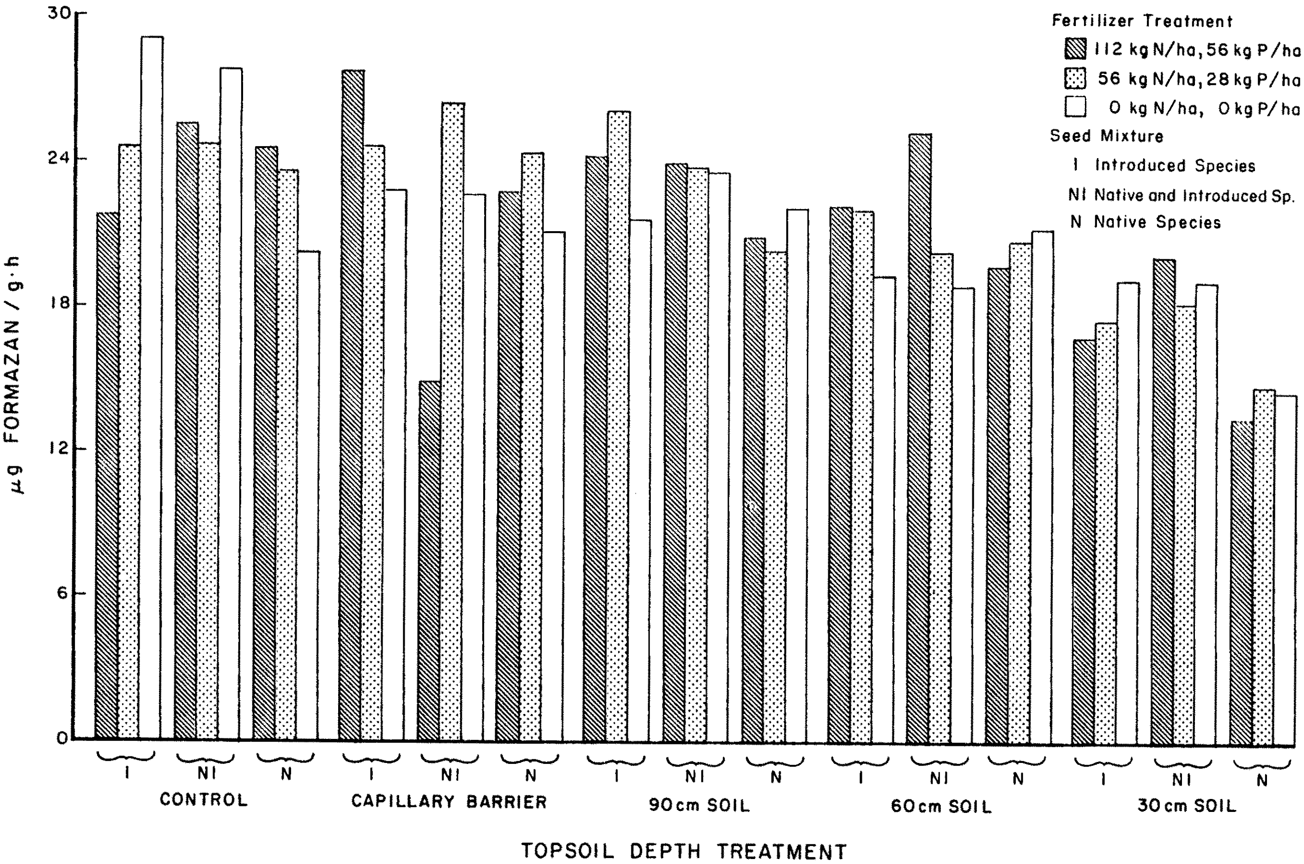

Fig. 17. Dehydrogenase activity with glucose amendments for the Retorted Shale Successional Study, considering topsoil depth, seeding mixture, and fertilizer effects--June 1981.

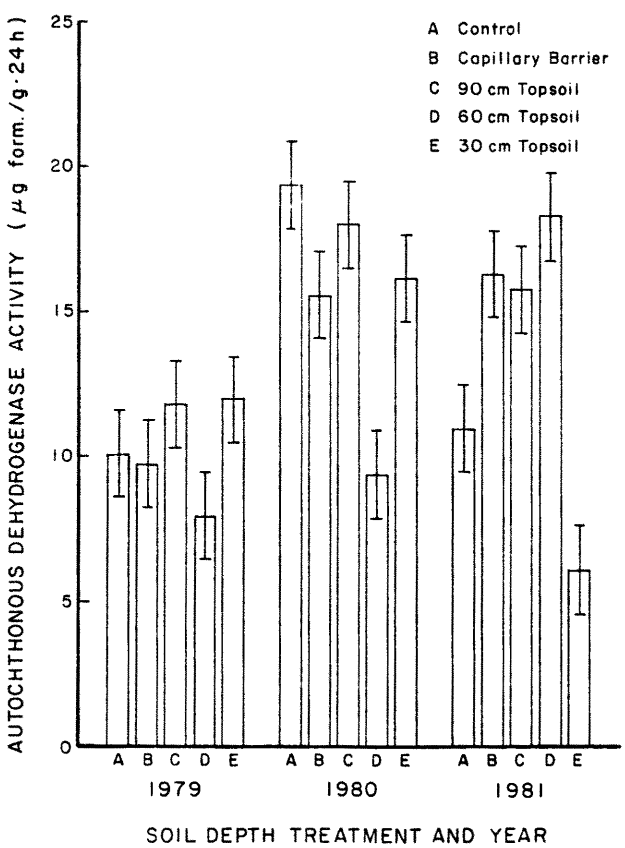

Fig. 18. Dehydrogenase activity without glucose amendments on the Retorted Shale Successional Study--1979, 1980, and 1981 responses.

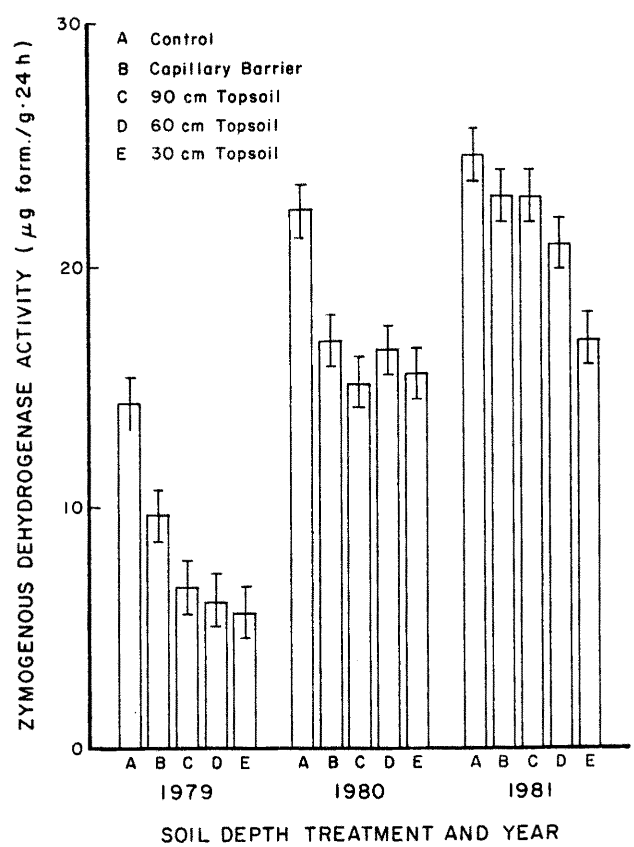

Fig. 19. Dehydrogenase activity with glucose amendments on the Retorted Shale Successional Study--1979, 1980, and 1981 responses.

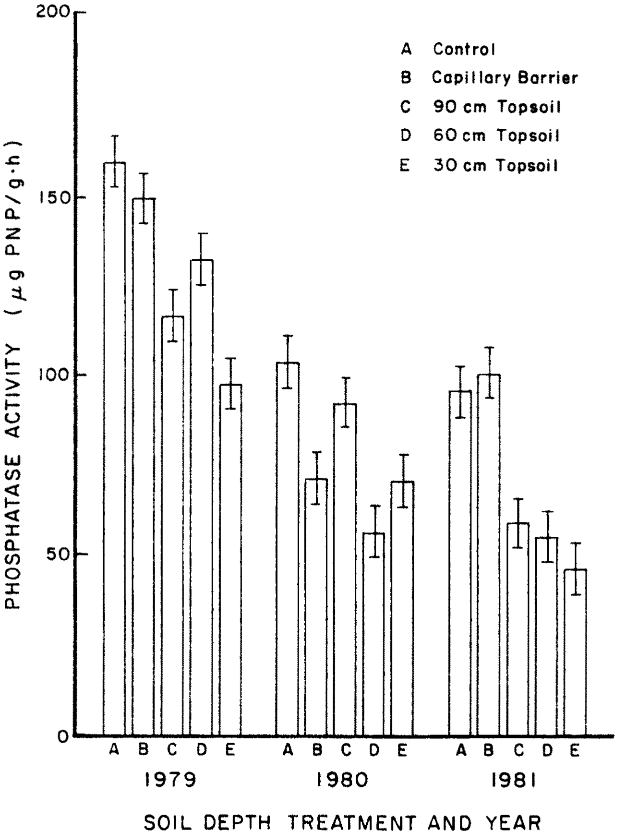

Fig. 20. Phosphatase activity on the Retorted Shale Successional Study--1979, 1980, and 1981 responses.

reduction seemed to be independent of soil depth over the shale and might be related to soil moisture dynamics and plant growth. A significant increase in acetylene reduction activity was observed in the soil over the capillary barrier. Results from the 1980 sampling showed the soils 30- and 60-cm deep to be the most severely reduced in activity, with higher activity in the soil 90-cm deep and in the soil over the capillary barrier. All of the replaced soils were significantly lower in activity than the disturbed control in the 1980 sampling.

An interaction between seed mixture and fertilization was observed in 1979, while in 1980 only a simple effect of fertilizer on acetylene reduction occurred. The highest average acetylene reduction rate is associated with the higher fertilizer addition and the lowest rate with no addition of fertilizer (Fig. 23). This is a change from earlier responses where added minimal nitrogen led to a repression of nitrogen fixation potential, possibly due to changes in plant-soil relations.

A similar effect of fertilizer was again observed in samples taken in June 1981, in that fertilization led to increased nitrogen fixation potentials, although the lower fertilizer level had the higher activity (Fig. 23). All of the treatments of soil over retorted shale had average activities which fell well within the 95% confidence intervals for the control area activity (Table 12) for May and June of 1981.

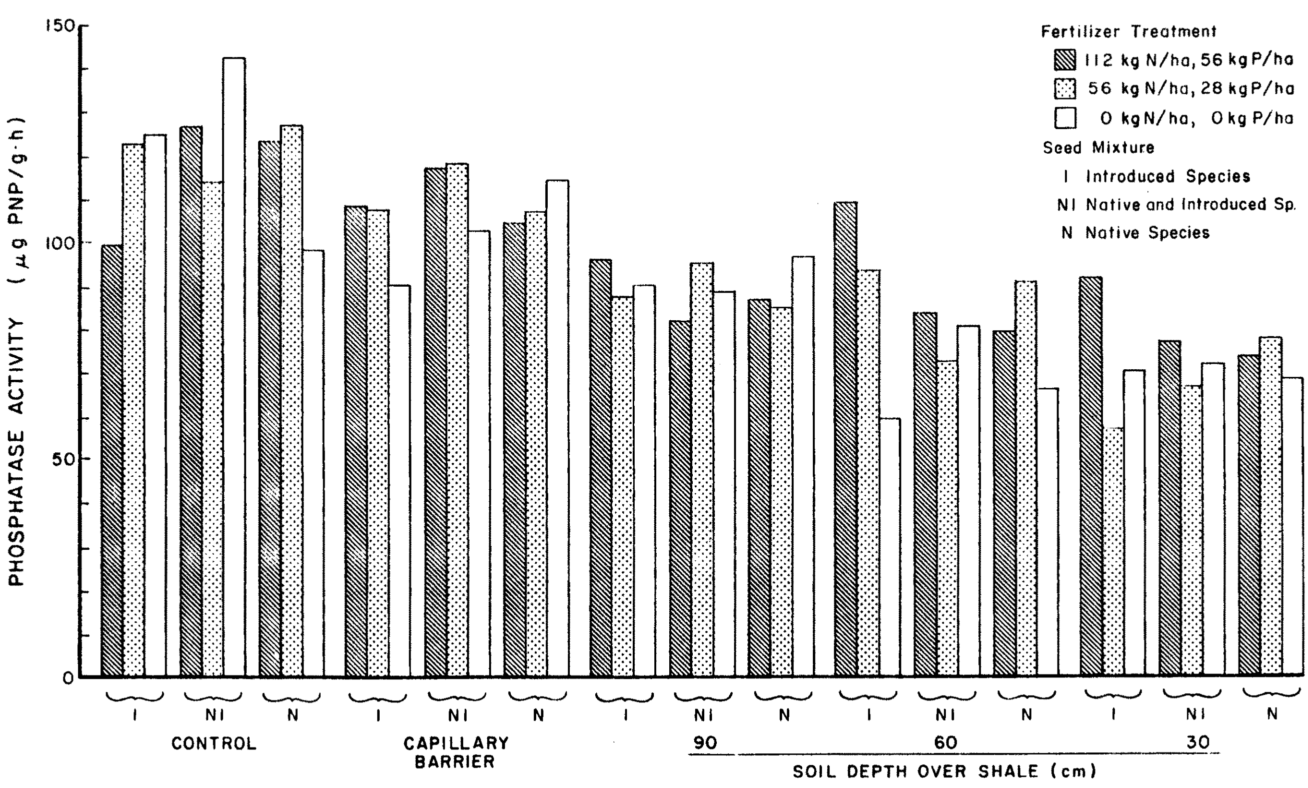

Fig. 21. Phosphatase activity on the Retorted Shale Successional Study, considering seeding mixture and fertilizer effects on combined 1979, 1980, and 1981 responses.

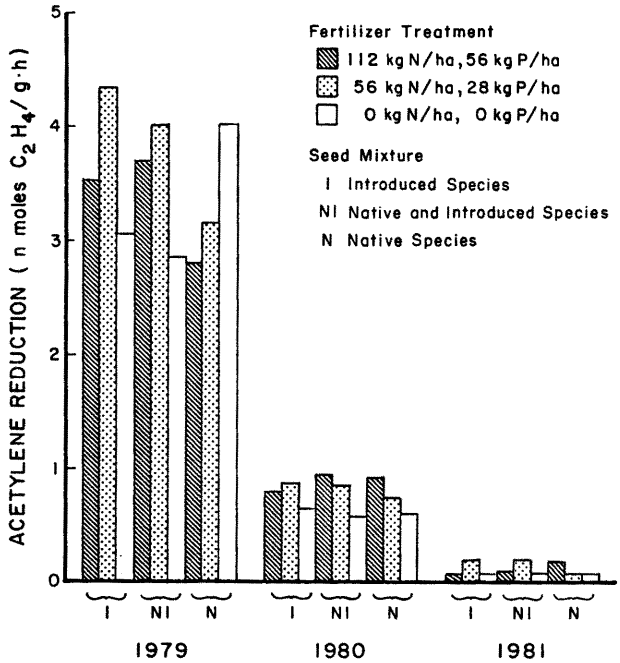

Fig. 22. Nitrogen fixation potential for the Retorted Shale Successional Study--1979, 1980, and 1981 responses.

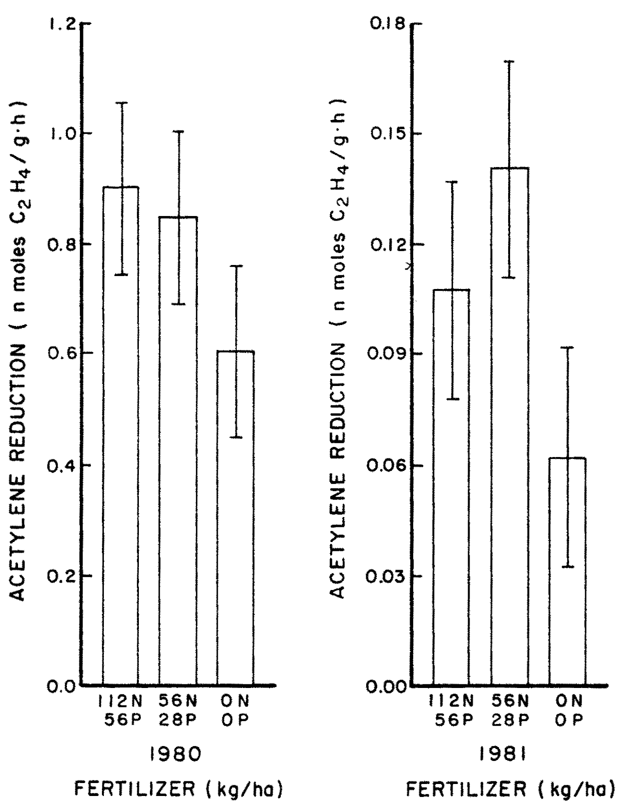

Fig. 23. Fertilization-nitrogen fixation potential interactions for the Retorted Shale Successional Study--1980 and 1981 analyses.

Denitrification Potential

The average denitrification potential for each of the treatment plots is shown graphically in Fig. 24 using samples taken in June. The disturbed control had the highest values, but the treatment with soil 90-cm deep over processed shale was not significantly lower than the control. Replaced soils at 60- to 30-cm deep over processed shale and the capillary barrier treatment had denitrification potentials which were less than half that of the disturbed control or the soil 90-cm deep over retorted shale.

The denitrification potential did show some reponse to fertilization, with the higher fertilization treatment having a higher denitrification potential than the lower level and no fertilization (Fig. 25). The lower fertilization level had an intermediate average denitrification potential which was not significantly different from either the plots with high or no fertilization.

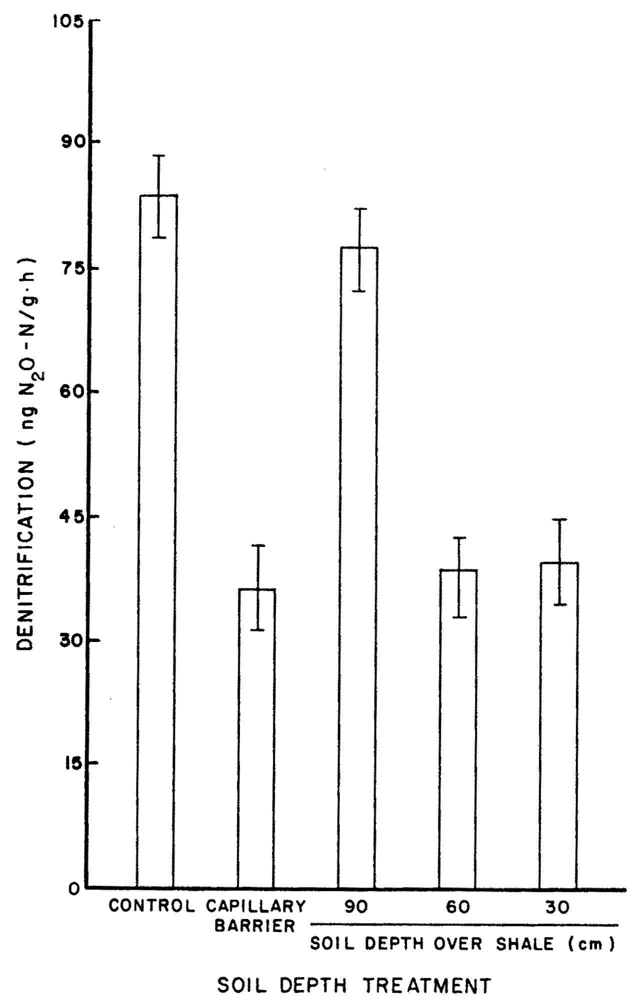

Fig. 24. Denitrification potential for the Retorted Shale Successional Study--1981.

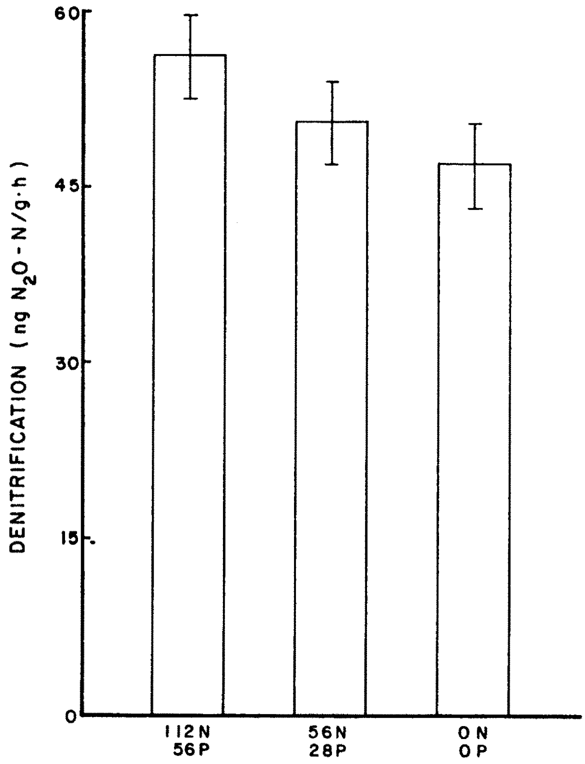

Fig. 25. Fertilization effects on denitrification potential for the Retorted Shale Successional Study--1981.

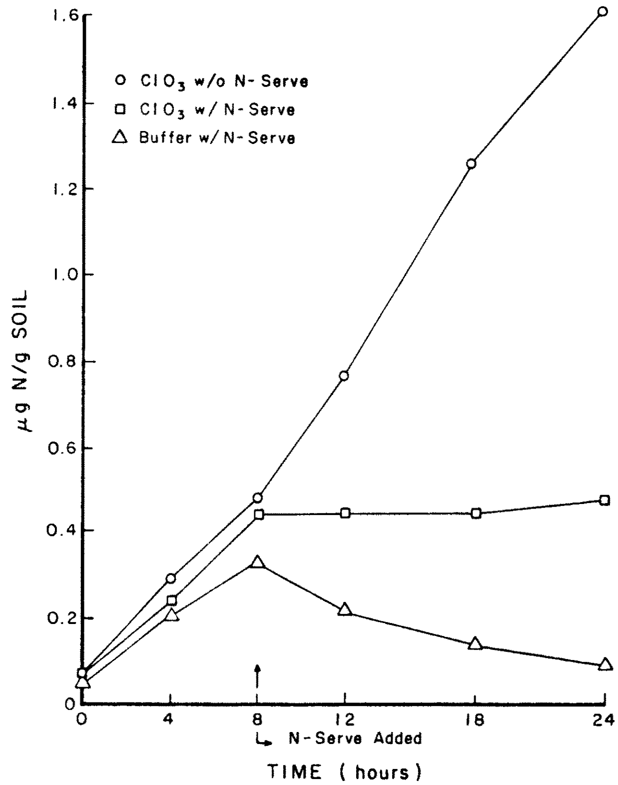

Fig. 26. Chlorate level effects on inhibition of nitrite oxidation.

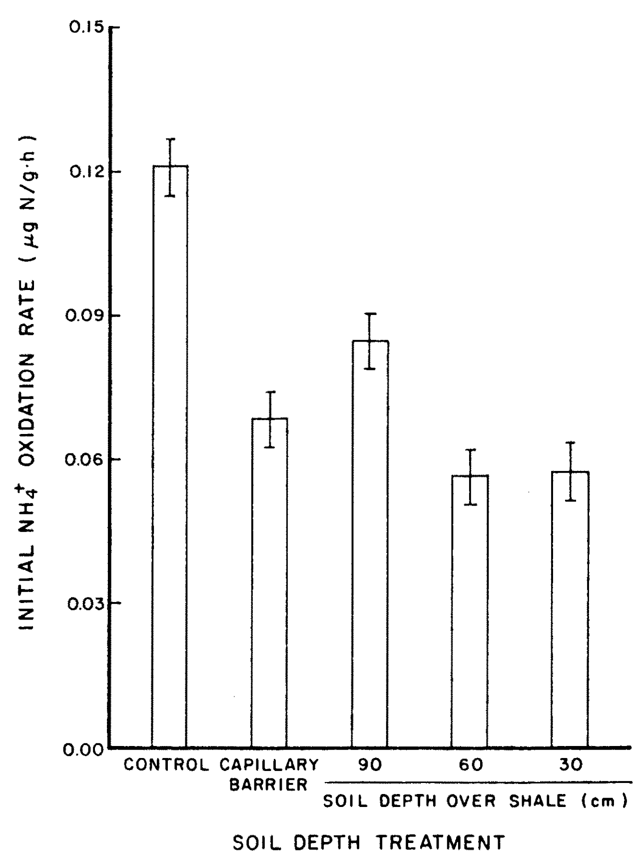

Fig. 27. Ammonium ion oxidation rates for the Retorted Shale Successional Study--July 1981.

Ammonium Ion Oxidation

The ammonium ion oxidation rate was assayed using techniques that should indicate the "initial" status of the microbial population capable of carrying out this reaction by adding low enough concentrations to ensure that ammonia toxicity would not develop. The incubation times were kept short (30 hours) to preclude appreciable growth of the nitrifier population. An experiment was carried out to ensure the effectiveness of the chlorate block in inhibiting the oxidation of nitrite to nitrate (Belser and Mays 1980) in Fig. 26. Nitrapyrine at 10 mM ClO_3 resulted in cessation of nitrite accumulation. If nitrapyrine is added to the same system but without chlorate being present, the nitrite concentration decreases steadily as it is oxidized to nitrate. Belser and Mays (1980) point out that the blockage of nitrite oxidation by chlorate is not absolute and depends somewhat on the concentration of nitrite. However, if nitrite concentrations are kept low, inhibition of the oxidation is almost complete.

The highest average rate of ammonium ion oxidation was found in the disturbed control treatment (Fig. 27). All of the replaced soil treatments over retorted shale had lower oxidation rates, with the 90-cm deep soil over shale being significantly higher than the other replaced soils including the soil over the capillary barrier. The ammonium ion oxidation rate in the 60- and 30-cm depth of soil over retorted shale averaged less than half that of the disturbed control soil.

The initial ammonium ion oxidation rate response to fertilization indicated that where more ammonium has been added, the oxidation rate is higher, a pattern similar to that observed for the nitrogen fixation potential.

Compared to the ammonium ion oxidation rate measured from soils collected from the undisturbed control site June 2, 1981 (Table 12), all of the average rates from the treated plots are significantly lower.

Nitrite Oxidation

Nitrite oxidation was assayed using essentially the same techniques as were used for ammonium oxidation, except that ammonium ion and chlorate were not added, and nitrite disappearance was monitored using nitrapyrine to inhibit indigenous ammonium oxidation to nitrite. Based on experiments reported by Belser and Mays (1980), initially a concentration of 0.08 μM nitrapyrine was maintained to prevent ammonium oxidation; however, nitrate accumulation continued over periods longer than 24 hours. By doubling the concentration of nitrapyrine to about 0.17 μM no nitrite accumulation was detected in the slurries.

To demonstrate that the nitrite disappearance rate could be interpreted as the rate of oxidation of nitrite to nitrate, a low concentration of nitrite was added to two sets of shaker slurries of undisturbed control area soil; the concentration of both nitrate and nitrite was monitored over a period of 28 hours. To one set of slurries both nitrapyrine and ClO_3 were added to serve as a completely blocked control. The slope of the nitrate accumulation line and the nitrite disappearance line were essentially the same absolute value. By 28 hours of incubation the nitrite concentration in the undisturbed slurries was below detection. The 5-, 17-, and 23-hour data were used to calculate the coefficients for the least squares regression lines.

The nitrite oxidation rate from the soil depth treatments are shown in Fig. 28. There was a statistically significant effect of the interaction between fertilization and soil treatments over retorted shale on nitrite oxidation. It is apparent that the rate of nitrite oxidation does not follow a consistent pattern in relation to fertilization in each of the soil over retorted shale treatments. Average rates of nitrate oxidation for all of the replaced soil, irrespective of fertilizer treatment, were significantly below the 95% confidence limit values for the undisturbed control area.

Ammonium Ion Volatilization

This experiment was completed to provide initial information on ammonia fertilizer losses by volatilization using two fertilizer addition levels, 375 mg $(NH_4)_2SO_4$-N/l and 100 mg $(NH_4)_2SO_4$-N/l. No ammonia volatilization was observed from the soil treated with 100 mg $(NH_4)_2SO_4$-N/l after the first week. Total losses of ammonia from this treatment were very low (Fig. 29). In comparison, soil treated with 375 mg $(NH_4)_2SO_4$-N/l continued to lose ammonia for about

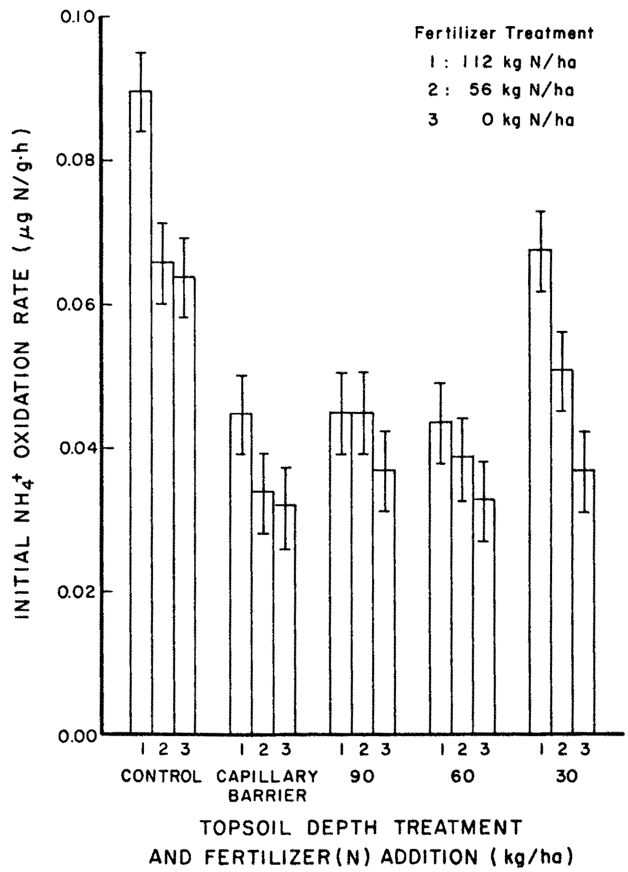

Fig. 28. Initial nitrite oxidation rate for the Retorted Shale Successional Study--July 1981.

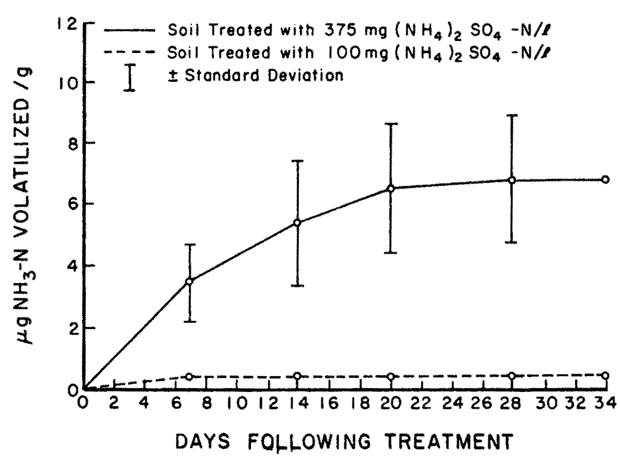

Fig. 29. Ammonium ion volatilization from Piceance Basin soil with two levels of added ammonium ion--1981.

28 days following treatment. At that time the soils had lost an average of 6.8 mg N/g dry weight of soil or about 8% of the NH_4^+-N originally added. The amount of ammonium ion added in the high treatment (about 84 µg N/g) corresponds roughly to a fertilizer application of 180 kg N/ha (assuming a 15-cm plow depth). The conditions of this experiment were conducive to nitrification, and at the end of the experiment all (94-113%) of the remaining ammonium ion nitrogen added had been oxidized to nitrate. These results suggest that volatilization may be a minor route for loss of ammonium ion added to these soils and relates to the continued observation of changes in nitrogen cycling processes three years after fertilization.

Soil Salinity and Ion Movement

One of the threats to successful revegetation of soil overlying retorted oil shale is the movement of salts and/or toxic elements from the shale upward in to the soil. Early observations (i.e., 1979) that soil enzymatic activity was suppressed in soils directly over retorted shale prompted an investigation into the possibility that soluble salts were moving into the soil from the retorted shale below, to the extent that surface microbial activity was being depressed. Cores taken June 3-4, 1981 were analyzed for soluble salt content as indicated by electrical conductivity of saturated paste extracts (Fig. 30).

The mean and range of conductivity in each of the core segments are plotted along with the average saturated paste extract conductivities measured in the topsoil or subsoil originally used to construct the plots in 1977.

Conductivity data from the 1981 cores show about the same level of salinity near the surface for all of the treated plots. However, in plots with soil depths of 60 and 90 cm, as the soil/shale interface is approached, some appreciable increases in salinity occurred. Apparently, between the two sampling times some salts from the processed shale have moved into the overlying soil immediately above the retorted shale on these treatments.

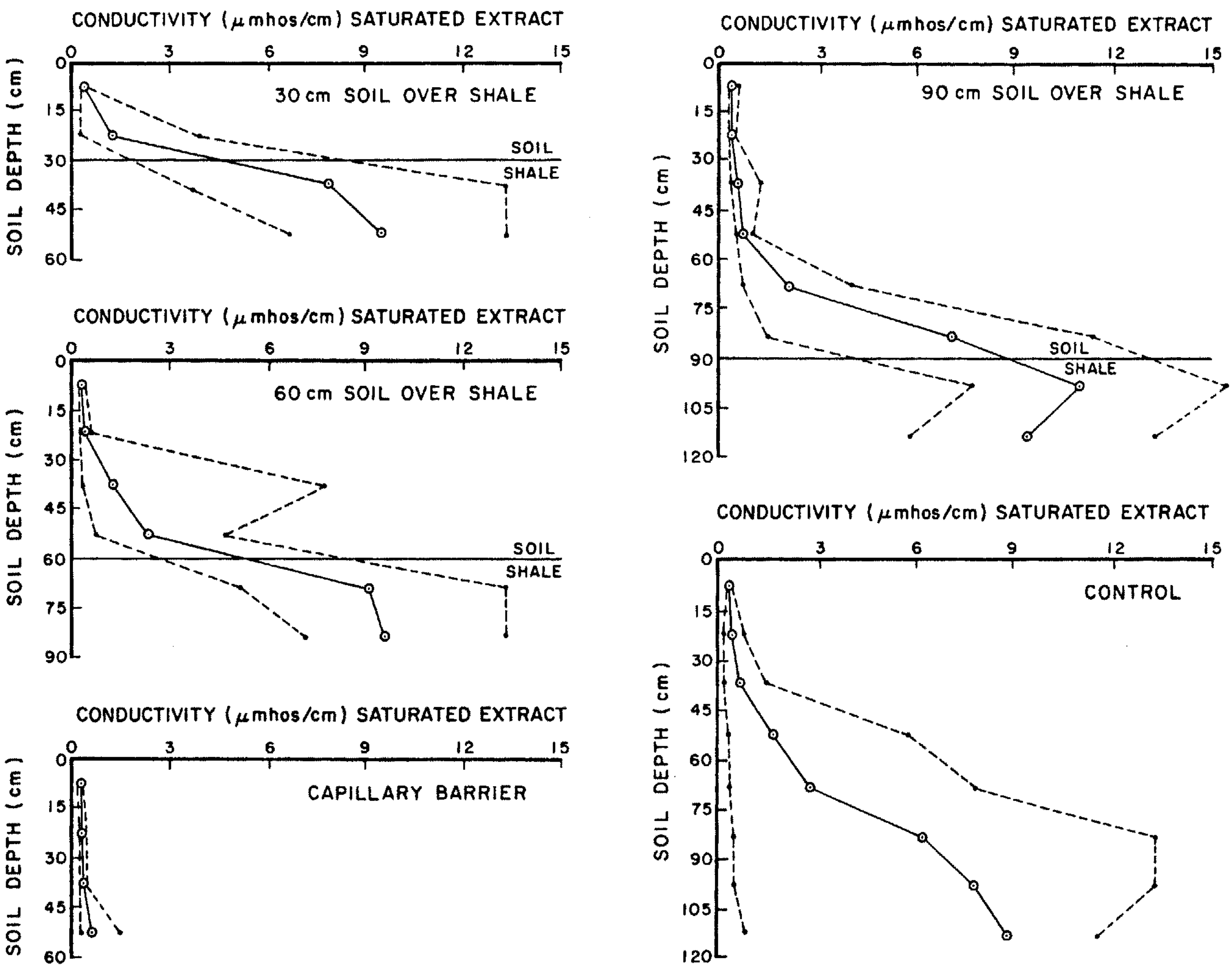

Fig. 30. Electrical conductivity of soils with depth across the shale interface for the Retorted Shale Successional Study--1981.

Several saturated paste extracts from soil overlying spent shale that showed high salinity were analyzed for specific major cations, anions, and elements known to be present in relatively high concentrations in retorted shale (Table 13). These data indicate that sodium, probably as sodium sulfate (sulfate was not analyzed), is the major cation moving from the shale into the soil where these samples were collected. The mobility of boron, molybdenum, and thiosulfate with 90 cm of surface soil are perhaps of equal interest to the major cations and anions. Elevated levels of molybdenum in plants whose roots have penetrated into retorted shale at this site have been measured previously by Lindsay et al. (1981).

Despite this movement of salts, molybdenum, boron, and reduced sulfur into the lower depths of the soil in certain locations, no evidence was found for increases in salinity near the soil surface where decreases in soil biological activity have been observed. These results suggest that processed shale perhaps interacts with the biological processes of the surface soil by mechanisms other than simple salt movement into surface soils.

Direct Counts of Bacteria

The results of direct miocroscopic counts of FITC stained bacteria in composited samples from subplots on each of the treatment plots are shown in Fig. 31. A significantly lower average number of bacteria was observed in the soils 90- and 30-cm deep over the retorted shale in comparison with the disturbed control treatment. The important point to be taken from these observations is that both microbial biomass and activity appear to be influenced by the presence of the oil shale components and that this trend is most evident with a 30-cm soil cover.

Depth Studies

For 1981, analyses of depth effects on microbial populations and activities were completed, including analyses of samples through the soil-shale interface. The dehydrogenase with glucose-treated samples and phosphatase showed essentially no activity when the shale had been

Table 13. Elements and ions in selected saturated paste extracts of high conductivity soils directly over retorted shale in 1981.

Surface Soil Depth Over Shale (cm)	Sampling Depth (cm)	Conductivity (mmhos/cm)	Conc. µg/ml in Saturated Paste Extract				
			Na	B	Mo	F	$S_2O_3^=$
30	15-30	3.9	732	0.3	0.5	1.0	NA†
60	45-60	4.7	986	1.7	1.5	0.8	<5
90	60-76	4.1	1000	2.5	0.6	1.6	37
90	76-90	10.4	2960	1.7	3.4	1.0	<7

†NA = data not available.

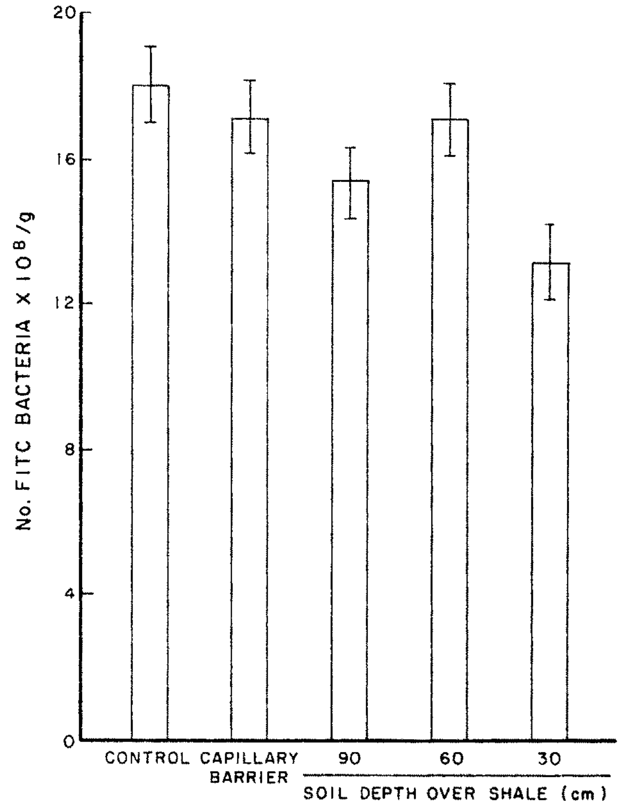

Fig. 31. Microscopic bacterial responses for the Retorted Shale Successional Study--July 1981.

reached. In contrast, the control dehydrogenase activities (without added glucose) in shale showed 15-30 µg of formazan production/g · hour in the shale. At this depth the values were equivalent to those found in the control soils. This would indicate that the autochthonous organisms had begun to colonize the shale resulting in increased biological activity. The viable bacteria were found at $2x10^6$/g in the shale. Distinct increases were also observed for the viable fungal propagules. The appearance of fungi in the first shale zone may also reflect the physical movement of soil spores into this zone. Further work on biomass development will be required to provide information on the significance of this observation.

Stored Soil Experiment

For 1981, when the plant material on the northern half of the stored topsoil pile had developed and matured, distinct differences in the soil dehydrogenase across the entire bore depth of 300 cm were observed. The background or zymogenuous dehydrogenase activity showed this most distinctly (Fig. 32), although a less distinct trend was shown for the glucose-amended dehydrogenase. In this case, the possible presence of plant materials

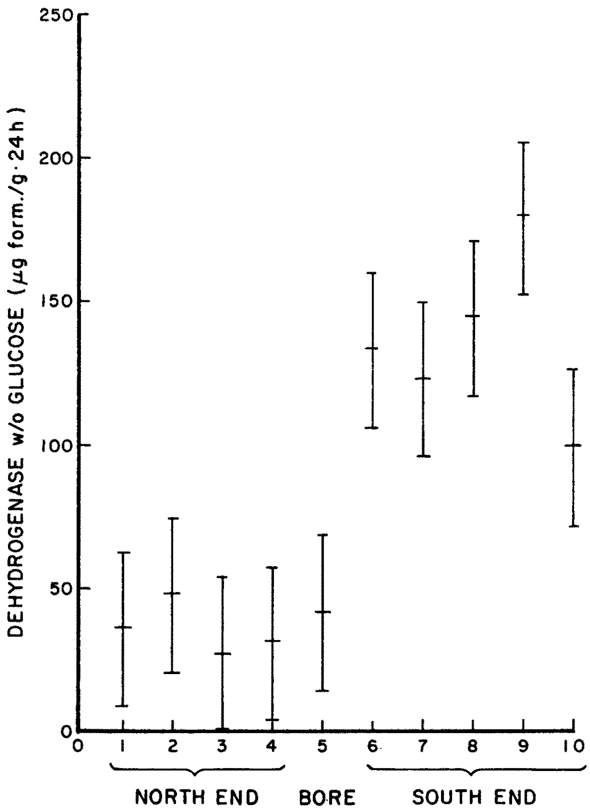

Fig. 32. Dehydrogenase responses without glucose for the Stored Soil Experiment--1981.

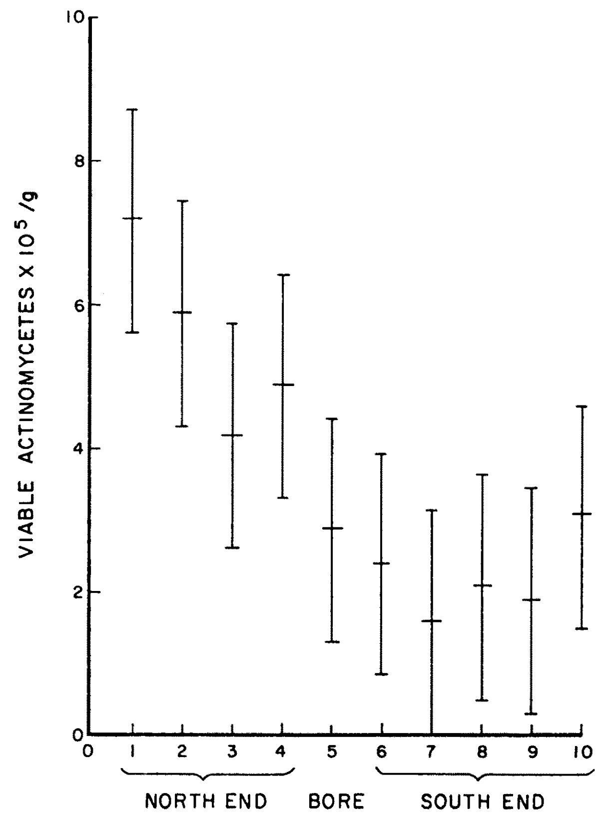

Fig. 33. Viable actinomycete populations for the Stored Soil Experiment--1981.

served to maintain activity at a significantly higher level. A similar trend was shown for phosphatase activity. The mycorrhizal responses at this plot are discussed by Reeves et al. (this report).

When comparing the depth effects over all bores, higher increased ATP and dehydrogenase activities with glucose were observed in the 0-25 cm soil zone. No other major differences occurred with depth over the balance of the stored soil volume.

In contrast to the higher biological activity in the planted north end of the stored soil pile, the viable counts indicated a different set of relationships. Under these conditions higher numbers of actinomycetes and fungi were found in the unplanted south end of the pile, a trend which is also supported by the bacterial data (Fig. 33). In addition, the southern portion of the stored soil system also had a higher soil water content. These results suggested that relationships between microbial populations and activities may undergo marked shifts with plant development.

Plot Analyses With Commercial Companies

Colony TOSCO II

The Colony TOSCO II species plots established in 1970 involved the planting of nine single plants and one mixture on 60 cm of topsoil over spent shale and the planting of a species mixture on spent shale amended with sawdust and phosphorus and also on spent shale mulched with crushed oil shale, talus, jute, or straw. The plots were leached and irrigated with approximately 100 cm of water over a two-year period (Bloch and Kilburn 1973). In addition to microbial activity, biomass and below-ground development were evaluated. Only dehydrogenase with glucose showed a significant response with depth of sampling, with the maximum activity occurring near the surface in the 0-10 cm depth. No differences in pH, soil moisture, dehydrogenase without glucose, or nitrogen fixation were observed.

The TOSCO II mulched and fertilized plots were designed to evaluate the effects of phosphorus fertilizer, sawdust, and other mulch treatments on the development of plant communities. As would be expected, the dehydrogenase activity was again highest in the surface soils. In relation to the sawdust and phosphorus amendments, the major observation was that increasing amounts of phosphorus fertilizer did not lead to corresponding increases in biological activity.

Anvil Points

These plots (established in 1973) were designed to evaluate the effects of variations of shale materials (TOSCO and USBM) on plant growth with shale-to-surface, 15 cm, and 30 cm of soil over shales in comparison with only soil (control),

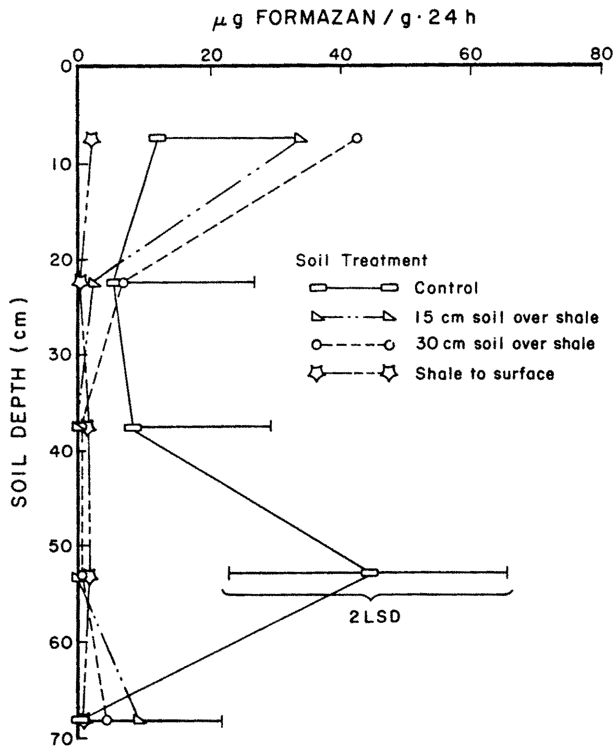

Fig. 34. Surface soil depth effects on dehydrogenase with glucose in relation to sampling depth for the TOSCO plots, Anvil Points--1981.

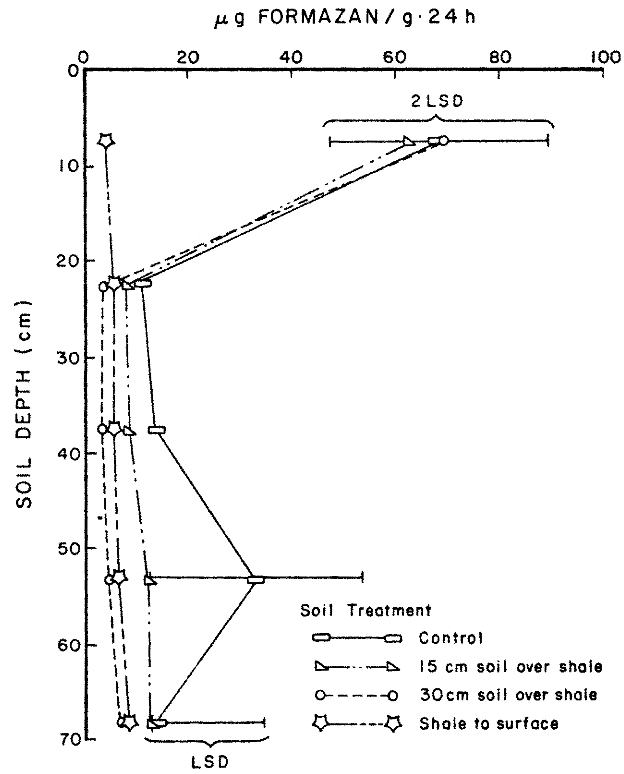

Fig. 35. Surface soil depth effects on dehydrogenase without glucose in relation to sampling depth for the TOSCO plots, Anvil Points--1981.

using both south- and north-facing slopes. The plots were leached with 100 cm of water prior to seeding and fertilized with N and P. Irrigation water was also applied for plant establishment. For specific details on methods see Harbert and Berg (1978). As would be expected, the major increases in carbon processing activity (dehydrogenase) occurred in the surface soil zone, with a direct relationship being shown between surface soil presence and activity. This set of relationships was shown most clearly with the glucose amended dehydrogenase (Fig. 34). The dehydrogenase without glucose gave equivalent responses with all amounts of added soil over the TOSCO materials (Fig. 35). The USBM shales used at this site showed similar dehydrogenase responses across the plots (both with and without glucose amendments), and the shale-to-surface had the lowest activities.

Piceance Basin

This set of plots (established in 1973) was designed with TOSCO and USBM materials in a manner similar to those used for the lower elevation Anvil Points plots (Harbert and Berg 1978). Similar analyses of dehydrogenase activity and nitrogen fixation potential were completed. Dehydrogenase with added glucose showed a distinct depth effect with 15-cm and 30-cm surface soils over spent shale and also with shale to the surface. The major finding was that with USBM shale to the surface a higher activity was detected than with the TOSCO materials. The data also indicated a significant effect of depth and of surface soil on dehydrogenase, with the USBM material showing a significantly higher formazan activity in the surface soil zone, being 30.8±19.1 for the TOSCO shale and 83.7±5.6 μg of formazan for the USBM shale.

Similar relationships were seen for the dehydrogenase with added glucose (again with the USBM showing higher rates of activity than the TOSCO shale). No significant differences in nitrogen fixation potential were observed between the two shale types at this location, although a depth effect was observed. In addition, a slope aspect effect on nitrogen fixation potential was observed in the surface soil zone, with the west-facing slope showing a significantly higher activity than the east-facing slope. These results indicate that temperature and moisture differences between east and west slopes may have significant effects on the development of some biological processes.

Union Shale

This set of plots involved both the Process B (established in 1975) and the Union Decarbonized (established in 1974) processed shale materials. Soil depth (15 and 30 cm), including shale-to-surface as a variable, and aspect using 25% (west) and 50% (north) were variables used in this study. The Process B and decarbonized plots were fertilized with N and P and leached with 36 cm of water prior to seeding and irrigated with 39 cm of water for plant establishment (Berg et al. 1979; Heron et al. 1980). The Union Process B shale, in comparing controls, 15 cm and 30 cm of surface soil over shale alone and with the shale-to-surface, did not show differences in relation to pH and moisture, although the shale-to-surface did have a lower dehydrogenase activity. The soils over shale had lower values than the soil control plots both with and without carbon amendments, although these were not statistically different (Table 14). The N_2 fixation potential showed similar responses, but with rather large variations around the means it was not possible to draw statistical inferences from these trends.

The Union Decarbonized plots were also monitored for moisture, pH, dehydrogenase (control and glucose-amended), and nitrogen fixation potential. In relation to the variables of shale cover (control, 15-cm and 30-cm soil over shale, and shale-to-surface) and sampling depth, the shale-to-surface had uniformly higher water contents at all sampling depths. The shales to surface had pH values in the range of 11.5-11.6, while the soils over shale had pH values of about 8.7 and the control soil pH values averaged 8.1. For both dehydrogenase assays expected responses were observed, in that with decreasing soil over shale decreased dehydrogenase activities occurred in comparison with controls. The major limitation of these assays was the lack of sufficient replications to strengthen the statistical conclusions which could be drawn from these results. The nitrogen fixation potential data showed similar trends, in that with the presence of shale to surface markedly decreased activities were observed.

These studies, if repeated in the future, should be carried out using additional replications. After six years it is evident that distinct decreases in decomposition activities do occur in surface soils placed over retorted shales, even with revegetation and plant growth.

Lurgi Shale-Soil Interactions

During the research period initial studies of Lurgi shale effects on surface soil microbial processes were carried out. The Lurgi retorting process results in a fine textured, gray, spent shale. As the spent shale serves as a heat transfer carrier, most of the carbon residues are oxidized. The Lurgi retorted shale obtained from the Rio Blanco Company was used in this study and had the following physical characteristics: bulk density of 0.75 g cc^{-3}, particle density of 2.67 g cc^{-3}, % porosity of 72%, and pH of 11.7. Lurgi shale was mixed with a control soil from the Intensive Study Site at various concentrations (5, 10, and 25% with appropriate soil and shale controls) and incubated in a humidified environment at 25° for seven weeks. The percent moisture was held at approximately 10% by weight. Ammonium ion oxidation rate, phosphatase, and dehydrogenase activities were monitored.

The changes in physical characteristics of the surface soil and Lurgi shale mixtures are summarized in Table 15. With increased additions of shale, the bulk density decreased while the percent porosity and pH increased. The percent organic matter was much lower in the pure Lurgi shale, reflecting the higher processing temperature for this material. Addition of Lurgi retorted shale brought about significant reductions in microbial activity. There is a trend towards decreasing activity with an increasing concentration of shale, except in the glucose-amended dehydrogenase with a high Lurgi shale concentration (Table 16). Preliminary experiments indicte that the higher formazan production is due to chemical reduction of the tetrazolium electron acceptor by retorted shale components rather than by biological processes. Chemical reduction of tetrazolium compounds has been described previously (Casida et al. 1964).

SUMMARY AND CONCLUSIONS

These studies have been directed towards better understanding microbial aspects of plant-soil systems established on disturbed soils, with or without underlying shale materials, in relation to the following variables:

- -- Degree of soil disturbance
- -- Soil depth over shale
- -- Soil storage
- -- Role of a capillary barrier in protecting soil from underlying salt-containing materials.

Table 14. Effects of plant growth, surface soil presence, and depth of sampling on dehydrogenase activity for both Union Process B shale plots.

	Surface Treatment			
Sampling Depth (cm)	Control	15 cm Soil Over Shale	30 cm Soil Over Shale	Shale-to-Surface
0-10	61.6 ± 7.4	42.3 ± 28.2	48.4 ± 4.6	4.2 ± 1.5
10-20	26.4 ± 1.7	2.4 ± 0.9	3.0 ± 0.7	2.6 ± 1.4
20-30	32.5 ± 32.3	3.1 ± 1.3	12.8 ± 10.5	3.5 ± 2.5
30-40	26.0 ± 6.0	2.1 ± 1.3	0 ± 0	2.6 ± 1.0

Table 15. Physical characteristics of topsoil and Lurgi retorted shale mixture.

Soil-Shale Mixture	Bulk Density (g cc^{-3})	Particle Density (g cc^{-3})	% Porosity	pH	% OM
Control soil	0.90 ± 0.05	2.3	60.87	7.87	1.3 ± 0.16
5% Lurgi	0.91 ± 0.0	2.31	60.61	8.78	1.2 ± 0.11
10% Lurgi	0.94 ± 0.01	2.39	60.67	9.39	1.4 ± 0.07
65% Lurgi	0.89 ± 0.04	2.38	62.61	10.7	1.3 ± 0.33
100% Lurgi	0.74 ± 0.06	2.67	72.08	11.74	0.7 ± 0.06

Table 16. Ammonium ion oxidation rate, phosphatase, and dehydrogenase activities in surface soil-Lurgi retorted shale mixtures.

Soil-Shale Mixture	Ammonium Ion Oxidation		Phosphatase		Dehydrogenase Activity			
					Unamended		Glucose-Amended	
	μg N g^{-1} h^{-1}	% Decrease	μg PNP g^{-1}	% Decrease	μg Formazan g^{-1} 24 h^{-1}	% Decrease	μg Formazan g^{-1} 24 h^{-1}	% Decrease
Control soil	0.21	--	86.65 ± 9.4	--	24.65	--	30.22 ± 5.8	--
5% Lurgi	0.05	75	59.2 ± 5.6	25	17.45 ± 0.76	25	12.3 ± 2.0	57
10% Lurgi	0.001	99	53.2 ± 7.1	28	1.84 ± 0.04	92	1.6 ± 0.8	94
25% Lurgi	0.004	97	22.2 ± 5.7	66	2.47 ± 0.48	86	45.28 ± 9.8†	--
100% Lurgi	0.005	--	1.44	--	1.42	--	11.79 ± 35.6†	

†Chemical reduction of TTC in the presence of glucose.

-- Microbial interactions with fertilization, mulching and organic residue additions, plant types, and irrigation.

The objective of these studies was to allow soil microbial information to be used in the development of reclamation strategies, and for monitoring plant-soil system reestablishment after disturbance. Based on these information needs, critical aspects of microbial biomass and functioning have been studied including key steps in carbon, nitrogen, and phosphorus cycling.

These studies have shown that different degrees of soil disturbance will have long-lasting effects on belowground processes and that with better maintenance of the surface soil and decomposer subsystems such effects can be minimized. To have better plant responses in suboptimum materials after more extreme disturbance, it may be necessary to modify the microbe-soil system to better reestablish plant functions. Studies of rhizosphere microbial responses which are planned for the next research period should provide needed information bearing on this point.

Regarding the monitoring of carbon cycling, it is apparent that the glucose-amended and control dehydrogenase assays are monitoring responses of different microbial populations. The background organism activity, which may be more dependent on the availability of native soil organic matter, did not show the panel effects given by the glucose-amended assay. The clear effects of panel treatments and shale presence suggests a relationship to carbon availability and plant functioning, either direct or indirect.

Together with the carbon cycle monitoring, the nitrogen process monitoring which was carried out during this research has shown that a single fertilizer treatment at the beginning of reclamation of a disturbed site can have long-lasting effects. The results of the nitrification and ammonium volatilization trials also suggest that only a minor part of the added N will be lost due to volatilization.

After three years distinct effects of fertilization (positive) and shale presence with decreasing depths of surface soil (negative) were observed involving ammonium ion and nitrate levels and nitrification, denitrification and nitrogen fixing processes.

The nitrogen fixation potential, which initially (1979) showed negative responses with added nitrogen, now shows increased responses which suggests that the belowground plant development which has resulted from fertilizer additions has exerted a more dominant effect on the nitrogen fixation potential.

The broadest conclusions from the Retorted Shale Successional Study for future reclamation planning involve the changes in activity over time and the electrical conductivity measurements. The longer-term trends towards decreases in nitrogen fixation potential and phosphatase activity, coupled with increases in dehydrogenase activity over time, suggest that a major shift in biogeochemical cycling has occurred with reestablishment of a more mature plant community. This may reflect the reallocation of nitrogen and phosphorus to less available pools and the subsequent decrease in cycling of these components. In contrast, the increase in carbon in the systems with plant root development may be related especially to the increases in the zymogenous dehydrogenase activity. It will be important to monitor these trends in the next several years to determine when the systems may reach equilibrium in relation to the disturbance and retorted shale variables which are being tested in this study.

A major point of design of the capillary barrier in a surface soil protection system is to prevent the movement of salt into overlying soil layers. These results indicate that the capillary barrier has served this purpose, as no increase in conductivity occurred with increased soil depth over the barrier. In contrast, the other treatments (soil directly over shale) without the capillary barrier show increases in conductivity with increased depth. The major point is that the surface soil zone, where biological responses were observed in relation to soil depth and shale variations, did not show increased conductivity values. This suggests that the underlying shale materials may interact with surface soils by means other than or in addition to salt movement. It will be essential to better understand the nature of these interactions to effectively manage disturbed soil placed over these types of materials. This may be more critical when larger amounts of waste material are covered by similar shallow soil layers.

In summary, based on information obtained to date, the microbiological studies appear to be providing information which will improve and strengthen the potential for rehabilitation and reclamation of disturbed ecosystems in arid regions.

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IMPORTANCE OF MYCORRHIZAL FUNGI IN REVEGETATING DISTURBED SOILS AND RETORTED SHALE

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OBJECTIVES

From previous studies we have shown that most of the dominant plants in stable ecosystems in the Piceance Basin of Colorado and in the surrounding regions of the semiarid West are mycorrhizal, that is these plants have symbiotic vesicular-arbuscular mycorrhizal (VAM) fungi growing on their root systems (Reeves et al. 1979). These fungi greatly extend the effective root area of native plants and thus allow the plants to more effectively absorb nutrients and water from the soil.

When soil is severely disturbed or stored for long periods of time, either vegetation may be lacking or the invading plant species are typically nonmycorrhizal weedy species. Mycorrhizal fungi require suitable hosts to provide nutrients for their long-term survival; thus, the absence of suitable vegetation leads to significantly reduced populations of these beneficial fungi. When VAM fungi are eliminated by death, dilution of the soil with effectively "sterile" soil (deep soils), or sterilization (processed oil shale), those plant species that are mycorrhiza-dependent will not generally survive under natural conditions of low nutrient and moisture availability.

To understand the succession of plants that depend on these fungi and to optimize reclamation procedures on severely disturbed soils, information on the population ecology of these beneficial fungi is essential. The main goal of this subproject was to develop an understanding of how these fungi can be maintained or reestablished in disturbed, stored, or processed (e.g., oil shale) soils.

The specific research objectives for the past year were as follows:

1. Monitor Topsoil Storage Pile for changes in mycorrhiza inoculum potential (MIP) as a function of time and depth of soil.
2. Monitor Retorted Shale Successional Plots for changes in the MIP as a function of depth of replaced topsoil over retorted shale and in the underlying retorted shale
3. Monitor Annual Disturbance Plots and correlate changes in MIP with natural succession of native plants that have naturally invaded the disturbed areas.
4. Monitor Long-Term Fertility Plots for the effects of added nitrogen fertiizer on MIP.
5. Determine mycorrhizal status of pines and junipers native to the oil shale region of Colorado.
6. Determine the effects of different types of processed oil shale on the development of mycorrhizae.
7. Determine the mycorrhizal status of plants seeded in retorted shale and in topsoil over shale at selected industrial sites.

RESULTS TO DATE

Topsoil Storage Experiment

This study was begun in 1978 in order to monitor the changes in microbial processes associated with simulated topsoil storage conditions representative of those practiced by industry. The stockpile was constructed from the upper 30-50 cm of topsoil material found on the Intensive Study Site. The dimensions of the pile are 3 m high, 5 m wide at the top, and 23.5 m long with the sides and ends at the angle of repose.

During July 1978 and June 1979 the pile was sampled with a 7.5-cm diameter soil corer to a depth of 150 cm, and in July 1980 and August 1981 the pile was sampled to a depth of 270 cm. Four sample bores were made in each of the years of 1978 and 1979; ten sample bores were made in each of the years of 1980 and 1981. For each bore the upper 30 cm was divided into two subsamples (i.e., the upper 15 cm and the next 15-30 cm). Further samples of the core were made at 30-60 cm, 60-90 cm, 90-120 cm, and 120-150 cm in 1978, 1979, 1980, and 1981. In 1980 and 1981 additional samples were made at 150-180 cm, 180-210 cm, 210-240 cm, and 240-270 cm. Soil for each subsample was sieved through a 1-cm sieve and analyzed according to the bioassay developed by Moorman and Reeves (1979). All bioassays were done in a growth chamber (d/n 14/10 hr, temperature 28/21°C, light approximately 350 $\mu Em^{-2}s^{-1}$) using pregerminated DeKalb XL 321 corn; bioassays were run for 21 days for each of the soil samples.

In 1979 one-half of the storage pile (north end) was planted with a mixture of native grasses, forbs, and shrubs; the other half (south end) was not planted and was manually weeded. Comparable data for 1978, 1979, 1980, and 1981 were obtained only from the nonplanted half of the pile. The results of the effects of seeding half the pile were obtained by comparing data from the two separate halves of the pile in 1980 and 1981.

Results and Discussion

The specific objective of this subproject was to determine if, during long-term storage, there are detrimental effects on the mycorrhiza inoculum potential (MIP) of the topsoil. The purpose of sampling different depths was to determine if greater depths offered greater or lesser protection of the MIP of the topsoil. Results of the change in MIP values over time and at different depths of the pile are given in Table 17.

A one-way ANOVA of MIP over the four years (1978-1981) shows highly significant reductions of MIP ($p<.001$). Regression statistics of this data give an $R^2 = 0.813$ for Linear Regression (MIP = MIP - 0.479 months where MIP_i is the initial MIP of the soil) and an $R^2 = 0.814$ for Exponential Regression ($MIP = MIP_i^{-.046\ months}$). The exponential equation best represents what is expected in that the death of the VAM fungal propagules is rapid at first, and a residual number are more resistant and survive for longer periods of time. In the upper 150 cm of stored soil no significant depth effect was obvious from the data because of the rather large variability among depths; this variability was the result of the heterogeneity of the soil at the time of construction of the pile. Fig. 36 illustrates the exponential curve for the death of the mycorrhizal fungi in the top 150 cm of the Topsoil Storage Pile.

When the 1981 MIP values (Table 18) of the upper 90 cm of soil (effective root zone) on the planted half of the pile were compared to the MIP values of the unplanted half of the pile, a highly significant difference was found ($p<.01$). These results indicate that seeding the stored topsoil significantly increased the MIP of the soil even though the MIP of the soil had decreased from the original levels present when storage began.

In 1980 and 1981 samples from deeper depths of the storage pile were obtained. In both years the deeper depths (150-270 cm) had larger MIP values

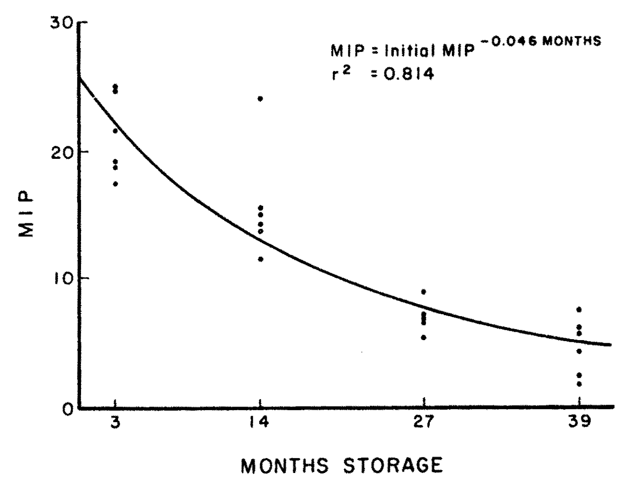

Fig. 36. Decrease in mycorrhiza inoculum potential (MIP) with time in stored topsoil at the Intensive Study Site.

Table 17. Mean MIP values for different depths of unplanted, stored topsoil.

Depth (cm)	Jul 1978	Jun 1979	Jul 1980	Aug 1981	$\bar{X}$
0-15	18.8	11.8	6.4	2.6	9.9
15-30	25.0	15.8	6.6	2.2	12.4
30-60	21.5	14.3	8.7	4.6	12.3
60-90	18.8	14.0	6.4	6.2	11.4
90-120	17.5	15.5	6.5	7.8	11.8
120-150	25.0	24.3	5.2	6.0	15.1
$\bar{X}$	21.1	15.9	6.6	4.9	

Table 18. Mean MIP values for the upper 90 cm (root zone) of soil from planted vs. unplanted halves of the Topsoil Storage Pile (1981) data.

Depth (cm)	Unplanted	Planted
0-15	2.6	41.3
15-30	2.2	39.4
30-60	4.6	35.2
60-90	6.2	15.0

than did the shallower depths (0-150 cm) of soil. These larger values are a reflection of the construction of the pile. When the pile was constructed, the upper layers of topsoil formed the base of the pile and the deeper layers of topsoil were placed over the upper layers. The MIP values from the deeper depths are given in Table 19.

Analysis of this data indicates that no significant change in the MIP values has occurred between 1980 and 1981. This is in contrast to the upper levels of the storage pile where significant decreases in MIP occurred between years 1978 and 1981. It should be emphasized that data for deeper depths were from only two years. Comparison of 1980-1981 data in Table 19 with the 1980-1981 data from the lower depths (60-150 cm) given in Table 17 indicates little change in MIP values at these lower depths. Although no significant depth effect was found for any year in the 1978 to 1981 data in Table 17, the current data in Table 19 suggest that there may be better preservation of the MIP of the soil at the lower depths. Further data from the 1982 sampling period will help resolve the nature of the changes in MIP at the lower depths of the storage pile. An analysis of both the 1980 and 1981 data indicates that there is a significantly higher mean MIP value for the 150-270 cm depth soil when compared with the 0-150 cm depth soil ($p<.05$).

Summary and Conclusions

Data over three years clearly indicate significant decreases in the MIP values of topsoil during storage especially when the soil is left unplanted. The nature of the death of the mycorrhizal fungi may be linear with a projected zero value at approximately 45 months' storage, or it may be exponential with a zero value projected at a somewhat later date. In order to refine the equation for the projection of the zero date additional samples will be taken in 1982.

When topsoil is planted with mycorrhizal plant species, the MIP values are maintained or increase significantly in the upper 90 cm of the pile. As yet, it is not known to what extent the root zone occupies the pile compared to the unplanted soil. As the roots of the planted species penetrate deeper into the pile, it is expected that the MIP values will increase. However, root penetration may be limited, thus the effective increase in MIP will be limited to the root zone.

The apparent "preservation" of the MIP in the lower depths (150-270 cm) may be an artifact of only two years' data or may be real. Additional sampling in 1982 will help determine which of these assumptions may be correct.

Table 19. Mean MIP values for the lower (150-270 cm) depths of the Topsoi Storage Pile.

Depth (cm)	1980	1981
150-180	8.3	8.3
180-210	9.9	9.5
210-240	12.0	13.7
240-270	13.3	16.0
$\bar{X}$	10.9	11.9

Recommendations

1. To preserve MIP values on stored topsoil, plant the topsoil with VA mycorrhizal species that are deep-rooted as soon as possible.

2. Avoid prolonged storage of topsoil. In a relatively short period (39 months) significant decreases in MIP occurrs; these decreases can exceed those found in severely disturbed soils.

3. Monitor storage piles for changes with time and depth. If unplanted or very deep, stored soil approaches a zero MIP value and the soil will be less suitable as a growth medium in establishing a stable community of mycorrhizal plant species.

Retorted Shale Successional Plots

This study was initiated in 1977 to test the effect of surface disposal plans for processed shale on plant growth and succession and on microbiological processes in the soil as well as in the retorted shale. Six plots, representing artificial profiles of soil over processed shale, were constructed. Vertical profiles of these plots (Fig. 37) are characterized as follows:

1. Retorted shale to surface
2. 30 cm topsoil over shale
3. 90 cm topsoil over shale
4. Check plot (no shale, only soil)
5. 60 cm topsoil over shale
6. 60 cm topsoil over rock capillary barrier over shale.

To analyze the MIP values of these plots representative subplots B1, B2, and B3 (three replicates of each) were selected. These subplots had previously been planted with a native grass-forb-shrub species mixture and fertilized at 112 kg N + 56 kg P/ha (B1), or 56 kg N + 28 kg P/ha (B2), or 0 kg N + 0 kg P/ha (B3). Each soil/shale treatment was sampled to a depth of 30 cm into the shale below the soil, with the exception of plot 6 which was sampled to the rock capillary barrier. Details of the initial treatments on these plots are found in Redente et al. of this report.

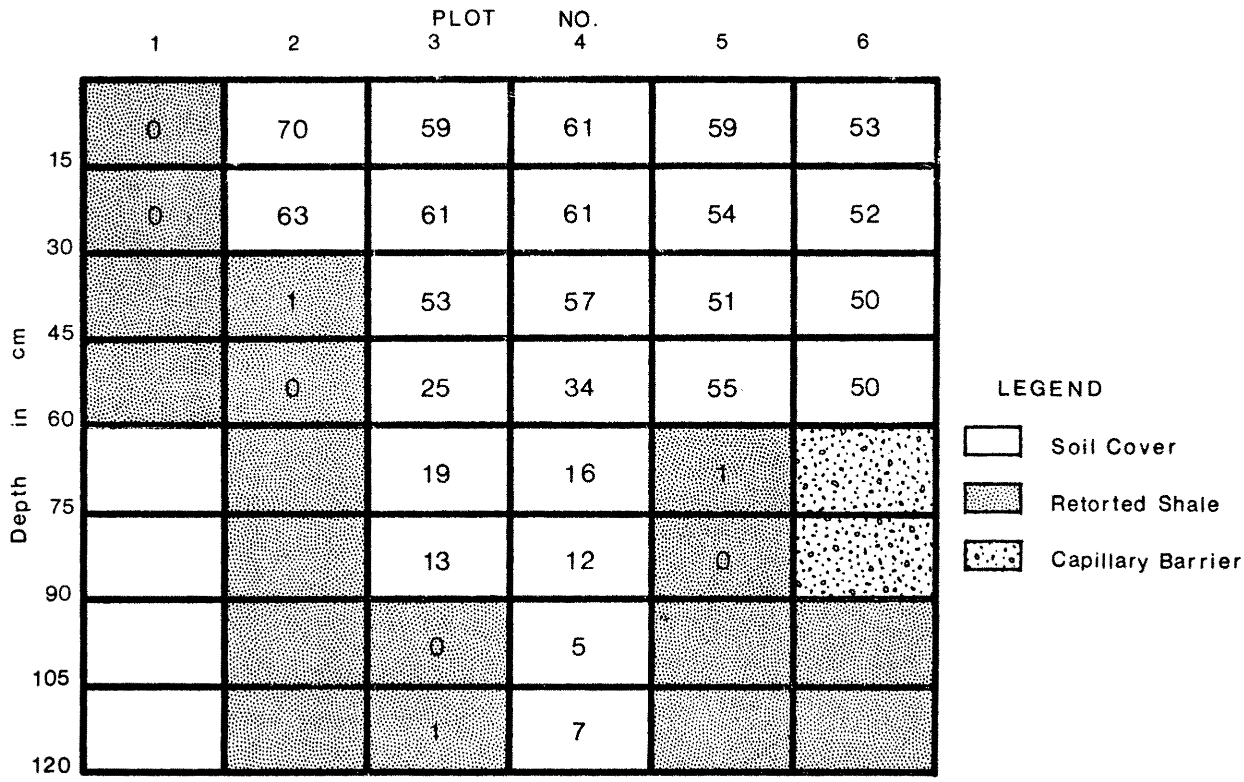

Fig. 37 Vertical profiles of Retorted Shale Successional Plots 1-6. Number in each rectangle is the mycorrhiza inoculum potential (MIP) of that soil or shale at the depth given on the left axis.

On June 3-4, 1981 the B1, B2, and B3 subplots were sampled using a 7.5-cm diameter soil corer. Soil and shale samples were each divided into 15-cm samples. In the lab each sample was sieved through a 1-cm sieve and analyzed for MIP using a slight modification of the bioassay (Moorman and Reeves 1979). Rather than using 9-cm square pots, tubular "Conetainers" (used in forest nursery practices) 3.5x21 cm were planted with pregerminated DeKalb XL 321 corn. The bioassay was run for 21 days in a growth chamber (d/n 14/10 hr, temperature 28/21°C, light approximately 350 $\mu Em^{-2}s^{-1}$).

Results and Discussion

Analysis of the 1981 data from the soil of subplots B1, B2, and B3, comparing equivalent depth samples, revealed no significant differences in MIP due to fertilizer effects, i.e., subplots B1-B3 were similar in their MIP values in the 0-45 cm of soil. Obvious significant differences (Fig. 37) were found when soil was compared to retorted shale at equivalent depths--the shale had an MIP = 0. The 1981 data support the 1980 data from these plots for fertilizer effects.

No significant differences due to depth effect were found in the 0-45 cm of comparable soils. Obvious significant differences were found when soil was compared to retorted shale at all equivalent depths. However, significant ($p<.01$) MIP values were found in the 45-60 cm zone in comparing Plots 3 and 4 with Plots 5 and 6 (Fig. 37). Plots 3 and 4 represent deeper soils, and the roots of the plants apparently continue to penetrate this soil. In contrast, at the 60-cm depth in Plots 5 and 6 the roots meet a "barrier zone", i.e., the oil shale or capillary barrier. Previous observations indicate that the roots tend to coil up in this region. The greater root volume in this zone would allow the mycorrhizal fungi to reproduce and effectively increase the MIP of the soil in this area. There is, then, an apparent "shale effect" in that the presence of shale inhibits the penetration of roots into this material and the shale does not contain viable mycorrhizal propagules.

The decrease in MIP values with greater depths (0-90 cm, Fig. 37) in Plots 3 and 4 is highly significant ($p<.01$). This decrease is consistent with the decrease reported for undisturbed soils of the Piceance Basin (Schwab and Reeves 1981).

Summary and Conclusions

Based on two years' data, there is apparently no adverse effect in terms of MIP of the soil that processed oil shale exerts on soil over shale. Indeed, in the shallower soils (60 cm) the MIP actually improved when compared to natural soil (cf. Plots 5 and 6 to Plots 3 and 4). This improvement, however, does not extend into the shale (Plot 5); the lower shale zones do not allow mycorrhizal roots to penetrate and thus does not become a suitable medium for mycorrhizal species. When compared to the normal condition that is represented by Plot 4, it is apparent that functional mycorrhizae do not form in the processed oil shale in any of the other plots (Fig. 37). The processed shale sublayers do not develop into an effective plant growth medium within the time frame of the study. This conclusion is further substantiated by the data from the industrial plots sampled during 1981 and presented later.

Recommendations

1. Use fertilizer treatments up to 112 kg N + 56 kg P/ha if these fertiizer treatments are beneficial to the establishment of the aboveground species.

2. Consider applications of subsoils over shale before applying thin layers of topsoil in order to increase root penetration and MIP to that level found in natural conditions.

Annual Disturbance Plots

This study was initiated in 1976 to test the effects of soil disturbance on natural succession and on microbial processes. At a mid-elevation big sagebrush community in the Intensive Study Site, four increasingly severe disturbances were created on replicate 6x8-m plots. The disturbances were:

Treatment 1: Vegetation scraped, topsoil left in place

Treatment 2: Vegetation scraped, topsoil ripped to 30-cm depth

Treatment 3: Vegetation scraped, topsoil and subsoil mixed to a depth of 1 m

Treatment 4: Vegetation scraped, 1 m of subsoil placed over topsoil buried 1 m deep

In 1976, 1977, and 1979 replicate plots representing the above treatments were established to observe the effects of the initial treatment each year on the MIP of the soil and how MIP values correlate with recovery of natural vegetation over time on these plots. No additional disturbances occurred following the establishment of each plot. For each treatment over successive years, three samples of the top 15 cm of soil were removed at each sampling date (November 1978, June 1979, July 1980 and January, March, and May 1981). At each sampling date the three samples of each plot were composited. The soil was mixed in the lab, and a bioassay (Moorman and Reeves 1979) was run for 15 days in a growth chamber (d/n 14/10 hr, temperature 28/21°C, light approximately 350 $\mu Em^{-2}s^{-1}$) using pregerminated DeKalb XL 321 corn.

Results and Discussion

As previously reported (Reeves et al. 1979), severe disturbance significantly reduces the MIP values of soil. On these plots the MIP values tend to decrease with increasing disturbance (Table 20).

An analysis of the 1976 and 1979 plots indicates highly significant ($p<.01$) differences in the MIP values of the soils. An analysis of variance for the 1977 plots indicates a possible ($p=.07$) difference in MIP values for all disturbance treatments.

Recovery values over time indicated all MIP values increased and the less severe the disturbance the more rapid the recovery. An interesting aspect of the data is that Treatments 3 and 4 showed substantial increases in the MIP values even though there were relatively few mycorrhizal plants found on these plots. Most of the cover on these treatments was Russian thistle (Salsola kali) which is a nonmycorrhizal species. Recent research (Schmidt and Reeves, in press) indicates that S. kali may maintain VAM fungal propagules even though the plant remains nonmycorrhizal. Since this plant is one of the earlier invading weeds on severely disturbed soils in the semiarid West, this observation may prove to be highly significant. If S. kali can maintain or increase the MIP of disturbed soil, then this plant may prove to be beneficial in maintaining essential microbial activity following severe disturbance.

Details of the correlations of MIP values of the soils on these plots with aboveground vegetation was submitted for publication and is currently

Table 20. Mean changes in MIP values of Annual Disturbance Plots established in 1976, 1977, and 1979.

	Year of Sampling				
	1978	1979	1980	1981	$\bar{X}$
1976 Plots					
Treatment 1	23.0	55.0	66.5	62.3	51.7
Treatment 2	24.0	44.5	52.5	55.8	44.2
Treatment 3	4.0	13.0	11.0	28.0	14.0
Treatment 4	3.5	11.5	20.0	16.0	12.8
1977 Plots					
Treatment 1	9.5	49.5	39.0	25.2	30.8
Treatment 2	1.0	21.0	7.5	24.5	13.5
Treatment 3	9.5	16.5	2.5	10.2	9.7
Treatment 4	6.5	10.0	5.0	20.8	10.6
1979 Plots					
Treatment 1		33.5	48.5	37.8	39.9
Treatment 2		59.0	49.5	47.8	52.1
Treatment 3		29.0	18.0	23.0	23.3
Treatment 4		3.8	1.0	10.0	4.9

in revision (Doerr et al.) and should be published in the future.

Summary and Conclusions

Mild disturbances, as represented by Treatments 1 and 2, reduced the MIP of the soil, but the MIP values typically show good recovery within two years following disturbance. Severe disturbances, as represented by Treatments 3 and 4, significantly reduce the MIP of semiarid soils, and recovery of MIP values are much slower when compared to less severe treatments. Salsola kali, a nonmycorrhizal species, appears to have the capacity to maintain and perhaps increase the MIP of disturbed soils. Other related species in the Chenopodiaceae, e.g., Atriplex and Ceratoides, may also have this ability and would serve as excellent revegetation species on disturbed soils. The relationship of nonmycorrhizal species in maintaining VAM fungi on disturbed soils should be carefully studied in order to determine their potential for insuring essential microbial processes.

Recommendations

1. Determine the species of VAM fungi that can survive on the severely disturbed habitats and consider using these species in revegetation programs on severely disturbed soils.

2. If adequate natural seed and/or rhizome sources are present, relatively minor disturbances (equivalent to Treatments 1 and 2) are expected to recover within a few years without additional input of fertilizers.

3. Relatively major disturbances (equivalent to Treatments 3 and 4) must be reseeded and, perhaps, fertilized if a stable ecosystem consisting of mycorrhizal species is required.

4. All recommendations assume adequate moisture following disturbance. Long periods of drought will alter succession on disturbed soils.

Long-Term Fertility Plots

These plots were initiated in 1977 to determine the long-term fertility requirements for establishment and growth of plants on soil over shale. Disturbed topsoil (60 cm) was placed over Paraho processed oil shale (60 cm), and a uniform application of 130 kg P/ha was broadcast over the plots. Subplots received various nitrogen applications ranging from 0-448 kg/ha/yr, or up to 1793 kg/ha initial application, or various levels of sewage sludge as an initial application. Each subplot was replicated three times and planted with a mixture of native species. Details of these plots are found in the 1981 progress report for this project.

The subplots selected for sampling were those receiving uniform P applications (130 kg/ha) and differing inorganic N applications (Plots 1-8) and three check (control) subplots (Plot 20) with no additional N (Table 21). Sampling (three 15-cm deep, 7.5-cm diameter cores/plot) was done in October 1980 and March 1981. Soil was returned to the lab, and a 21-day bioassay was run in growth chambers using the methods previously described.

Results and Discussion

The specific objective of this subproject was to determine if there are trends of detrimental effects on the MIP values of disturbed topsoil over shale when moderate to high rates of nitrogen fertilizer are applied. Results from other labs on the effects of added fertilizers on mycorrhiza formation are variable--some experiments indicate that fertilization decreases mycorrhiza formation (Hayman 1975, Gerdemann 1975).

The general trend in these experiments indicates that annual incremental additions of N tend to decrease the MIP values of the topsoil and that the greater the amount of N added the greater the decrease in MIP. Table 21 gives the results of these experiments.

The generally lower MIP values found in the 1981 samples as compared to the 1980 samples were expected. The soil was sampled in late March 1981 before the plants had begun active growth, whereas in 1980 the soil was sampled at the end of the growing season (October).

As reported in the 1981 Progress Report, there appeared to be a fundamentally different response in MIP of the soil between large, initial applications of N (Plots 5-8) and smaller, incremental yearly additions of N (Plots 1-4). The combined data of 1980 and 1981, the mean MIP values, of the plots is given in Fig. 38. The addition of

Table 21. Mean MIP values of selected Long-Term Fertility Plots (disturbed topsoil over processed shale) receiving various amounts of nitrogen. All plots had a uniform application of phosphorus (130 kg/ha) in 1977.

Plot	Nitrogen Treatment	MIP Value Oct 1980	Mar 1981	$\bar{X}$
1	56 kg/ha/yr†	73.0	39.0	56.0
2	112 kg/ha/yr	54.0	40.7	47.4
3	224 kg/ha/yr	28.0	11.7	19.9
4	448 kg/ha/yr	26.0	29.3	27.7
5	224 kg/ha, initial application	65.0	35.7	50.4
6	448 kg/ha, initial application	74.0	46.7	60.4
7	896 kg/ha, initial application	56.0	37.7	46.9
8	1792 kg/ha, initial application	64.0	41.0	52.5
20	0 kg/ha	74.0	62.7	68.4

†Plots 1-4 received fertilization in these quantities each year for four years (1977-1981).

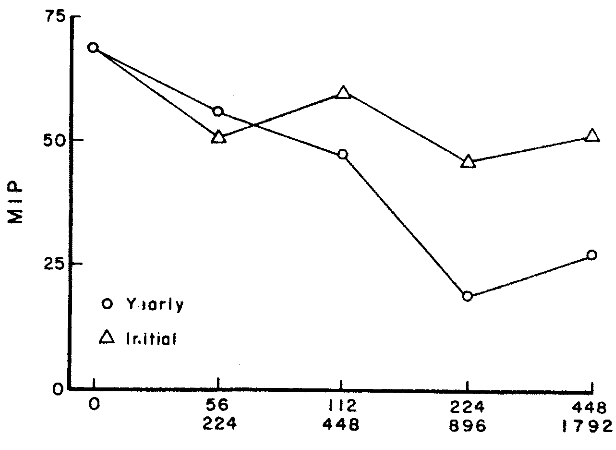

Fig. 38. Changes in mean mycorrhiza inoculum potential (MIP) on Long-Term Fertility Plots as a function of added nitrogen fertilizer.

nitrogen reduces the MIP of the soil, and yearly applications depress MIP to a greater extent than initial, high levels. However, even the heaviest N application did not appear to depress the MIP to a critically low level.

In 1980 we found significantly (p<.05) different MIP vales among Plots 1-4 where N fertilizer was added as yearly increments. Data for this year did not support this conclusion because of the extreme variance found in Plot 2. In 1980 we found no significant differences in MIP within Plots 5-8. This lack of difference is supported by the 1981 data. However, when Plots 1-4 (yearly additions of N) are compared to the check plots (Plot 20), significant (P<.05) reductions in the heavily fertilized plots (Plots 3 and 4) were obtained.

Summary and Conclusions

The initial application of moderate to heavy (224-1792 kg/ha) amounts of N to disturbed topsoil over processed shale does not significantly affect the MIP values of the soil after three years. Yearly applications of N equivalent to 224 or 448 kg/ha significantly reduce the MIP values of disturbed topsoil over processed shale but do not reduce MIP values to levels considered to be critically low.

Recommendations

1. Nitrogen may be added to disturbed topsoil at yearly intervals up to a total of 112 kg/ha/yr to enhance reclamation procedures. Heavier yearly applications (224-448 kg/ha/yr) of N decrease MIP values of the topsoil.

2. Large initial applications of N (224-1792 kg/ha) may be added to disturbed topsoil immediately after disturbance with no apparent effect on MIP values after three years.

3. For nitrogen treatments (yearly increments or large initial applications) determine the minimum level necessary to insure successful species establishment for the maximum cost/benefit ratio.

Mycorrhizal Status of Pines and Junipers

Two studies were initiated in 1981 to determine the mycorrhizal associations of pinyon and juniper trees native to the Piceance Basin of Colorado. These species occupy approximately 35% of the Piceance Basin and are the characteristic species of the Pinyon-Juniper Woodland type (Terwilliger et al. 1974). The objective of the first study was to determine the nature of the mycorrhizae in these species. The objective of the second study was to determine if inoculation with known ectomycorrhizal fungi could enhance the growth and development of pine seedlings as compared with noninoculated controls.

The nature of the mycorrhizae of *Pinus edulus* (pinyon pine) and *Juniperus osteosperma* (Utah juniper) was determined by examination of the feeder roots from excavated seedlings (N=20) with a maximum height of one-half meter. The roots were fixed, cleared, and stained with 0.1% trypan blue in lactophenol (Phillips and Hayman 1970) or embedded in parafin, sectioned, and stained.

To determine the effects of inoculation with ectomycorrhizal fungi, two fungi were selected for their wide host ranges and ecological adaptability. Mycelial inocula of *Pisolithus tinctorius* (isolate PT 133) and *Suillus granulatus* (isolate SG 75-20) were prepared in a vermiculite-peat moss medium. The controls were prepared exactly the same way except no fungal inoculum was added. After extensive growth of the fungus, each inoculum was leached to remove the excess nutrients and to encourage mycorrhizal development (Molina 1979).

The growth medium was made by mixing each inoculum separately with additional sterilized vermiculite-peat moss. Cone-shaped containers were filled with 150 ml of the medium. One pregerminated and surface-treated pinyon seed was planted per container. Forty-two seedlings per treatment were grown in a growth chamber (d/n 14/10 hr, temperature 26/19°C) for six months. A completely randomized experimental design was used.

After six months' growth the following parameters were measured: (1) height, (2) shoot weight, (3) root weight, (4) total weight, (5) stem diameter, (6) number of side branches, and (7) percent mycorrhizal short roots of the total short roots.

Results and Discussion

Both *P. edulis* and *J. osteosperma* were found to be predominantly endomycorrhizal. Pelotons, vesicles, and nonseptate intracellular hyphae were the endomycorrhizal structures observed. Arbuscules were observed in J. osteosperma. Ectomycorrhizae were also observed associated with both hosts but were not as common as endomycorrhizae; less than 5% of the short roots were infected with ectomycorrhizal fungi.

Members of the family Pinaceae have previously been considered only to form ectomycorrhizal associations whereas members of the family Cupressaceae form endomycorrhizae (Gerdemann 1968). Both P. edulis (Pinaceae) and J. osteosperma (Cupressaceae) formed a dual association with both ecto- and endomycorrhizal fungi, though both were predominately endomycorrhizal. This dual association may play a significant role in the ability of pinyon and juniper trees to become established in the semiarid regions of the Piceance Basin. The unique dual association offers an excellent experimental system to compare the beneficial effects of vesicular-arbuscular (VA) vs. ectomycorrhizae. To date these dual associations have not been examined experimentally.

None of the pinyon seedlings, either inoculated or noninoculated, became mycorrhizal in the six-month growth chamber study. Despite this, a one-way ANOVA revealed significant differences for root weight, shoot weight, and total weight ($p<.05$) in the inoculated seedlings vs. the controls. An LSD test was calculated to compare the differences in the means (Table 22). Both inoculated treatments were significantly different from the control in shoot and total weight. For root weight, only seedlings inoculated with S. granulatus were significantly different from the noninoculated controls.

It was not apparent why the seedlings were nonmycorrhizal. Several factors, including fertilization rate and the specificity of the ectomycorrhizal fungi for pinyon, may have influenced mycorrhiza development. Because none of the seedlings were mycorrhizal, the observed growth response cannot be easily explained. Possible hypotheses include: (1) seedlings became mycorrhizal early in the experiment and subsequently lost the association, (2) fungi altered the growth medium in some manner which enhanced growth, or (3) both.

Summary and Conclusions

Pinus edulis and Juniperus osteosperma possess a dual mycorrhizal association consisting of both ecto- and endomycorrhizal fungi. In younger trees (<30 years old) the predominant relationship involves VA mycorrhizal fungi. For pines this is a unique observation since all other reports indicate that the genus Pinus is strictly ectomycorrhizal. The predominant endomycorrhizal association in P. edulis may, in part, explain why the experiments with ectomycorrhizal fungi were not successful. Although junipers have been reported to be endomycorrhizal (Gerdemann 1968), no previous observations had been made on J. osteosperma. Understanding this uncommon dual mycorrrhizal relationship in these important species in the Piceance Basin may prove useful in reclamation programs for the Pinyon-Juniper Woodland type.

Many reports have demonstrated the requirement of mycorrhizal fungi for proper growth in gymnosperms (Marx 1975). Researchers have emphasized the need to select and tailor specific mycorrhizal fungi for specific sites in order to enhance reclamation (Marx 1975). To date little research effort has been placed on selecting those mycorrhizal fungi specifically adapted to semiarid environments. Additional research efforts in this area are needed.

Recommendations

1. Determine those species of endo- and ectomycorrhizal fungi native to the Piceance Basin that can be used to resynthesize mycorrhizae in pinyons and junipers.

2. Prior to transplanting use the selected mycorrhizal fungi to inoculate tree seedlings to enhance survival and growth.

Table 22. Pinyon pine growth response due to mycorrhial fungi. Columns with the same letter are not significantly different ($p<.05$).

Treatment	Height (mm)	Stem Diameter (mm)	Side Branch (numbers)	Shoot Weight (g)	Root Weight (g)	Total Weight (g)	Root/Shoot Ratio
Control†	62 a	2.7 a	2.7 a	0.63 a	0.56 a	1.20 a	0.892 a
PT 133‡	61 a	2.8 a	3.4 a	0.83 b	0.66 ab	1.47 b	0.841 a
SG 75-20§	61 a	2.8 a	3.1 a	0.84 b	0.71 b	1.55 b	0.902 a

†Controls were not inoculated with a mycorrhizal fungus.

‡PT 133, isolate 133 of Pisolithus tinctorius, used as the mycorrhizal inoculum.

§SG 75-20, isolate 75-20 of Suillus granulatus, used as the mycorrhizal inoculum

Effects of Different Shales on Development of Mycorrhizae

Due to time and space limitations the objectives of this study were not accomplished in time for the 1982 report. Future studies will begin in the late spring of 1982.

Mycorrhizal Status of Soils and Shales at Industrial Sites

These studies were initiated in June-July 1981 in order to determine the microbial processes associated with topsoils, topsoil over retorted shale, and retorted shales of different origins. The industrial reclamation sites investigated were near Rifle, Colorado. Sites sampled were Anvil Points (TOSCO II and USBM shales), Parachute Creek (Union Oil decarbonized and Process B shales), and Parachute Creek (Colony-TOSCO shales). The Anvil Points plots were constructed in 1973, the Colony plots were constructed in 1971, and the Union plots were constructed in 1975.

Soil, shale, and soil-over-shale samples were obtained using a 7.5-cm diameter soil corer in June-July 1981. Samples were returned to the laboratory and screened through a 1-cm sieve and analyzed for MIP values using a 19-day bioassay following the procedures outlined in the Retorted Shale Successional Plots section of this report.

These studies are complementary to the Retorted Shale Successional Plots established at the Intensive Study Site in 1977.

The major objective of this research was to determine if there were differences in leached and nonleached shales in terms of their ability to support mycorrhiza development and if shales differ in their ability to become functional "soils" in terms of supporting mycorrhizae.

Results and Discussion

Bioassays of all the samples have been run, but all the slides have not yet been read. We expect this information will be complete by the end of May 1982.

To date, approximately half of the samples have been analyzed and the results of these analyses are given in Tables 23, 24, and 25. Results from these tables indicate that TOSCO II and Union decarbonized shale do not support extensive mycorrhiza development. The decarbonized shale to surface has an MIP = 0, and it appears to completely inhibit mycorrhiza formation. The TOSCO II shale, when leached, allows mycorrhiza formation in the upper 30 cm but inhibits mycorrhiza formation at deeper depths.

Results from soil over processed shale are consistent with the results obtained on the Retorted Shale Successional Plots at the Intensive Study Site. Leaching of the TOSCO II shale allows

Table 23. Mean MIP values of topsoil-over-processed shale, and processed shale obtained from Union Oil Company valley revegetative stabilization research study site.†‡

Plot	MIP	Depth (cm)	Type of Medium
Control soil 1			
1-1	56	0-15	Topsoil
1-2	45	15-30	Topsoil
1-3	50	30-45	Topsoil
Control soil 2			
2-1	63	0-15	Topsoil
2-2	62	15-30	Topsoil
2-3	24	30-45	Topsoil
Shale (decarbonized)			
1-1	0	0-15	Processed shale
1-2	0	15-30	Processed shale
1-3	0	30-45	Processed shale
Shale (decarbonized)			
2-1	0	0-15	Processed shale
2-2	2	15-30	Processed shale
2-3	0	30-45	Processed shale
15 cm soil over shale			
1-1	61	0-15	Topsoil
1-2	0	15-30	Processed shale
1-3	0	30-45	Processed shale
15 cm soil over shale			
2-1	27	0-15	Topsoil
2-2	0	15-30	Processed shale
2-3	0	30-45	Processed shale
30 cm soil over shale			
1-1	63	0-15	Topsoil
1-2	42	15-30	Topsoil
1-3	41	30-45	Topsoil + shale§
30 cm soil over shale			
2-1	83	0-15	Topsoil
2-2	25	15-30	Topsoil
2-3	0	30-45	Processed shale

†Plots constructed in June 1975, 25% slope, west aspect.

‡Plots sampled in June 1981.

§Because of the angle of the plot and the soil corer some of the topsoil was mixed with the processed shale in this sample.

limited mycorrhiza development in the upper 30 cm if the shale extends to the surface. However, when leached shale is covered with topsoil, little mycorrhiza development occurs below the soil layer (Tables 24 and 25). It should be noted that after eight years leached TOSCO II shale does allow mycorrhiza development in contrast to no mycorrhiza development after six years on Union decarbonized shale (Table 23) or four years on unleached Paraho shale at the Intensive Study Site (Fig. 37).

Summary and Conclusions

The general lack of rapid mycorrhiza development in TOSCO II, Paraho, and Union decarbonized shale suggests that these shales are not suitable plant growth media for sustaining certain beneficial microbial processes. Leaching and exposure to the environment enhances the ability of TOSCO shale to support mycorrhiza development. Amendments (e.g., the addition of soils) to the shales may allow mycorrhiza development. Techniques for amendments with subsoils or topsoils need to be investigated to determine the level of amendments necessary to develop a suitable plant growth medium that will support mycorrhiza development in reclamation species.

Table 24. Mean MIP values of topsoil-over-processed shale, and processed shale obtained from Anvil Points revegetation study site.†‡

Plot	MIP	Depth (cm)	Type of Medium
Control soil 1			
1-1	51	0-15	Topsoil
1-2	39	15-30	Topsoil
1-3	20	30-45	Topsoil
1-4	21	45-60	Topsoil
1-5	14	60-75	Topsoil
TOSCO II shale (leached)			
1-1	84	0-15	TOSCO II processed shale
1-2	10	15-30	TOSCO II processed shale
1-3	1	30-45	TOSCO II processed shale
1-4	0	45-60	TOSCO II processed shale
1-5	0	60-75	TOSCO II processed shale
TOSCO II shale (leached)			
2-1	81	0-15	TOSCO II processed shale
2-2	11	15-30	TOSCO II processed shale
2-3	0	30-45	TOSCO II processed shale
2-4	0	45-60	TOSCO II processed shale
2-5	0	60-75	TOSCO II processed shale
15 cm soil over shale			
1-1	84	0-15	Topsoil
1-2	17	15-30	TOSCO II processed shale
1-3	0	30-45	TOSCO II processed shale
1-4	1	45-60	TOSCO II processed shale
1-5	0	60-75	TOSCO II processed shale
15 cm soil over shale			
2-1	76	0-15	Topsoil
2-2	19	15-30	TOSCO II processed shale
2-3	0	30-45	TOSCO II processed shale
2-4	0	45-60	TOSCO II processed shale
2-5	0	60-75	TOSCO II processed shale
30 cm soil over shale			
1-1	40	0-15	Topsoil
1-2	74	15-30	Topsoil
1-3	5	30-45	TOSCO II processed shale
1-4	0	45-60	TOSCO II processed shale
1-5	0	60-75	TOSCO II processed shale
30 cm soil over shale			
2-1	49	0-15	Topsoil
2-2	--§	15-30	Topsoil
2-3	0	30-45	TOSCO II processed shale
2-4	1	45-60	TOSCO II processed shale
2-5	0	60-75	TOSCO II processed shale

†Plots constructed in 1973, east aspect.

‡Plots sampled in June 1981.

§Insufficient sample size to obtain MIP data.

Table 25. Mean MIP values of topsoil-over-processed shale, and processed shale obtained from Anvil Points revegetation study site.†‡

Plot	MIP	Depth (cm)	Type of Medium
Control soil 1			
1-1	76	0-15	Topsoil
1-2	55	15-30	Topsoil
1-3	12	30-45	Topsoil
1-4	7	45-60	Topsoil
1-5	14	60-75	Topsoil
TOSCO II shale (leached)			
1-1	7	0-15	TOSCO II processed shale
1-2	0	15-30	TOSCO II processed shale
1-3	0	30-45	TOSCO II processed shale
1-4	0	45-60	TOSCO II processed shale
1-5	0	60-75	TOSCO II processed shale
TOSCO II shale (leached)			
2-1	51	0-15	TOSCO II processed shale
2-2	8	15-30	TOSCO II processed shale
2-3	0	30-45	TOSCO II processed shale
2-4	0	45-60	TOSCO II processed shale
2-5	0	60-75	TOSCO II processed shale
15 cm soil over shale			
1-1	47	0-15	Topsoil
1-2	32	15-30	Topsoil + processed shale§
1-3	0	30-45	TOSCO II processed shale
1-4	0	45-60	TOSCO II processed shale
1-5	0	60-75	TOSCO II processed shale
15 cm soil over shale			
2-1	69	0-15	Topsoil
2-2	22	15-30	Topsoil + processed shale§
2-3	0	30-45	TOSCO II processed shale
2-4	0	45-60	TOSCO II processed shale
2-5	0	60-75	TOSCO II processed shale
30 cm soil over shale			
1-1	54	0-15	Topsoil
1-2	62	15-30	Topsoil
1-3	0	30-45	TOSCO II processed shale
1-4	0	45-60	TOSCO II processed shale
1-5	3	60-75	TOSCO II processed shale
30 cm soil over shale			
2-1	41	0-15	Topsoil
2-2	36	15-30	Topsoil
2-3	21	30-45	Topsoil + processed shale§
2-4	0	45-60	TOSCO II processed shale
2-5	0	60-75	TOSCO II processed shale

†Plots constructed in 1973, west aspect.

‡Plots sampled in June 1981.

§Because of the angle of the plots and the soil corer some of the topsoil was mixed with the processed shale in these samples.

Recommendations

1. Union decarbonized shale does not support mycorrhiza formation; it is not expected that this shale will become a suitable growth medium for long-term survival of mycorrhizal plant species without input of fertilizer and moisture. Amendments with topsoil may enhance the ability of the shale to support many reclamation species.

2. Leaching, seeding, and exposure to the atmosphere allows TOSCO II shale to support mycorrhiza development near the surface (0-30 cm) of the shale). Lower depths (>30 cm) do not support mycorrhiza development and are not expected to become suitable growth media for mycorrhizal plant species. Amendments with topsoil appears to enhance mycorrhiza development of the shale.

3. Develop techniques for amending shales with topsoil and/or subsoils to elevate microbial processes beneficial to reclamation species.

4. Consider covering processed shales with additional depths of amended shale or subsoils to encourage deeper root penetration and associated microbial processes.

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ECOGENETIC VARIABILITY IN NATIVE SHRUBS RELATED TO THE REESTABLISHMENT OF VEGETATION ON DISTURBED ARID SHRUBLANDS

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OBJECTIVES

1. Evaluate the natural variation within species (especially shrubs) native to the Piceance Basin, Colorado.

2. Make recommendations regarding source materials which can be expected to give long-term, natural success on particular sites.

3. Evaluate the responses of shrub species already growing in an edaphic environment containing retorted shale.

METHODS

Populations of nine native species (six shrub, two grass, and one forb) have been examined for ecotypic variation in a common garden at the Intensive Study Site. Methods of collection, propagation, measurement and analysis are detailed in previous progress reports.

Several additional measurements were made during the 1981 growing season. For Utah serviceberry (Amelanchier utahensis), several populations of which flowered for the first time, the number of locules and number of seeds per locule were counted in 10 fruits from each plant. The number of seeds per fruit and per locule in plant species is often indicative of reproductive potential and reflects the sort of selection pressure on the populations. Also in A. utahensis branch angles and ratios of the lengths of branches were measured. Branch angles and lengths are important in that they are the main parameters in determining how the photosynthetic (productive) surfaces are arranged in space.

Leaf weights and leaf area of a sample of the A. utahensis and snowberry (Symphoricarpos oreophilus) garden plants were also measured during the 1981 growing season. The leaves were collected in the afternoon when under the most water stress, immediately weighed, and then placed in distilled water for 24 hours and weighed again. The difference between fresh and imbibed weight reflects the relative water potentials of the leaves. This measure, called the water saturation deficit (Slavik 1974), is calculated as follows:

$$\frac{\text{saturated weight - fresh weight}}{\text{saturated weight - dry weight}} \times 100(\%)$$

This gives the amount of water gained on imbibition as a percent of the total water content. A leaf that can take up more water presumably has an increased ability to get more water from the roots under drier conditions.

A new procedure was employed for winterfat (Ceratoides lanata). Near the beginning of the growing season the plants were clipped back to ground level. Recovery was measured several times thorughout the growing season by recording height and canopy diameters of individual plants.

For all the measurements made in the common garden, differences among populations of a species reflect some genetic differences. The population variants are called ecotypes. Our overall strategy for applying information on these ecotypes to reclamation depends on the argument that these genetic varieties have been selectively produced and maintained in their native situations. This means that particular source materials of the same species will be suited to particular reclamation sites that resemble the native site of the ecotype in question. Matching plant material to site suitability increases the probability of long-term, natural success.

To study the effects of retorted shale in the root environment other field studies were initiated. Field study of the responses of shrubs on existing soil-retorted shale combinations is somewhat constrained, since the study plots were designed by other investigators for other purposes. At the Intensive Site the Retorted Shale Successional Study plots were available. Of the various treatment combinations present, plants in one or two replicates of the native species seeding mixture and all of the fertilizer and profile treatments were measured. These plots were initiated in 1977.

In addition, plots in another part of the Piceance Basin, designed by Harbert and Berg (1974) for a study on vegetative stabilization of retorted oil shales, were used. These plots are on Wagon Road Ridge and between Black Sulphur and Ryan Gulches. The controls and every treatment combination of slope direction (north and south), type of shale (TOSCO and USBM, leached and unleached), and amount of soil cover (15 and 30 cm) were measured. Not every combination contained enough shrubs to measure reliably, and sampling had to be nondestructive. Plant height and the major and minor diameters of the canopy spread, assuming its shape could be approximated by an ellipse, were measured. The shrubs present in sufficient numbers to sample were big sagebrush (Artemisia tridentata) and fourwing saltbush (Atriplex canescens). An effort was made to find and measure 6-10 of the largest individuals of each species in every subplot where they occurred. The largest plants were chosen to avoid sampling younger plants established since the initial planting. One-way ANOVAs were used to test for different responses in plant size within the different treatments.

RESULTS

Ecotype Studies

The results from garden plantings for 1981 follow basically the same pattern as those of 1980. They are briefly described here. A few additional measurements were made for various species to explore aspects of adaptation not treated in previous years.

Prairie junegrass (Koeleria cristata) and Indian ricegrass (Oryzopsis hymenoides) were measured throughout the season for phenological advance, amount of reproduction (Table 26), plant height (height to the tip of the longest leaf or culm), basal diameter, and average leaf and sheath length. Differences among the populations of Koeleria cristata were significant for only four of the several measurements (Table 27)--plant basal diameter on 9 June, leaf length on 4 July, and diameter growth on 20 May to 9 June. These differences may also be biologically meaningful, but the results are too sparse to infer any general pattern of ecotypic variation. This species is known to vary ecotypically over Colorado (Robertson and Ward 1970). All of the populations of this species in the garden, however, are from the Piceance Basin. It is concluded that within this more limited geographic area, population materials are probably interchangeable as source materials for reclamation. The only qualification to this is that the population showing the most growth in diameter is the population native to the garden area itself. There still could be some site specificity.

The populations of Oryzopsis hymenoides were significantly different for virtually everything measured (Table 28). All the plants in the garden

Table 26. Definitions of the phenological stages of the grass species Oryzopsis hymenoides and Koeleria cristata in the ecotype garden and the index for the amount of reproduction. For each measurement date (see Table 27) the phenological stage and amount of reproduction for each plant was noted by recording the corresponding index number.

	Index
Phenological Stage	
Vegetative only	1
Inflorescence in boot	2
Inflorescence ≤ 1/2 out	3
Inflorescence > 1/2 out	4
Anthesis	5
Seeds present	6
Seed dispersal	7
Number of Reproductive Culms	
1 to 10	1
11 to 50	2
>50	3

Table 27. Koeleria cristata 1981 garden measurements. Results of one-way analysis of variance across four populations for each measurement and date combination.

Garden Measurement	F Value† May 20	May 29	Jun 9	Jun 15	Jun 24	Jul 4
Phenology	1.6	0.5	0.2	2.3	1.6	1.2
Reproductive culms	1.6	0.3	0.1	1.8	3.2*	0.7
Plant height	0.7	0.1	0.1	nm	nm	nm
Basal diameter	1.0	2.9	4.3**	nm	nm	nm
Leaf length	nm	nm	nm	nm	nm	3.5*
Sheath length	nm	nm	nm	nm	nm	0.8
Diameter growth May 20-Jun 9			5.0**			

†Significant F ratios are indicated: .05*, .01**; "nm" means "not measured".

Table 28. Oryzopsis hymenoides 1981 garden measurements. Results of one-way analysis of variance across 13 populations for each measurement.

Garden Measurement		F Value†
Average phenological stage:	May 25	2.0*
	May 29	3.2***
Reproductive culms:	May 29	5.0***
	Jun 9	2.3*
Plant height:	Jul 8	3.6***
Major diameter:	Jul 8	2.4*
Minor diameter:	Jul 8	2.2*
Basal area:	Jul 8	2.1*
Basal area x height:		2.4*

†Significant F ratios are indicated: .05*, .01**, .001***.

were more robust and fecund than plants growing in the surrounding native vegetation. However, in the garden some populations were significantly more robust than others. Some populations from outside the Piceance Basin (North Park) responded similarly to some populations native to the Basin. On the other hand, some Basin materials were more similar to populations from geographically remote areas (e.g., Florence, Colorado) than to other Basin populations. The prudent conclusion to make is that, for this species, site specific adaptations have occurred and are not likely to be interchangeable source materials among sites.

Scarlet globemallow (Sphaeralcea coccinea) was the only herb studied in the garden. It too is known from the literature to vary ecotypically, but our collections (all from within the Piceance Basin) do not generally differ significantly. In 1980 the only significant differences among populations were for petiole length. Of the three measurements made in 1981 (Table 29) only the number of flowers produced in the second flowering flush of the season seems to have biological importance. These plants would possibly have a higher reproductive potential. But again, there is not enough evidence here to conclude that there is important differentiation within the populations of this species.

For three of the shrub species, antelope bitterbrush (Purshia tridentata), true mountain mahogany (Cercocarpus montanus), and Atriplex canescens, the garden results do not provide evidence of important ecotypic differentiation. The results for Purshia tridentata (Table 30) show no significant interpopulation differences. The analysis for Cercocarpus montanus was presented in our 1981 progress report. Five populations of this species (three from the Piceance Basin and two from the east slope of the Colorado Front Range) showed slight differences in a few characteristics but overall had strong similarities. Two populations of Atriplex canescens from fairly similar sites were established initially with the hope of adding populations from different sites. This was not possible, thus no conclusions are drawn.

The species Amelanchier utahensis and Symphoricarpos oreophilus have received the most intensive study in the garden; more populations were collected and more measurements were taken. Both species show important ecotypic elaboration.

The garden results for Amelanchier utahensis in 1981 (Table 31) show significant population differences for most of the variables measured during the past two years. A few differences were not significant in 1980 but were in 1981; this could be due to the larger number of samples taken from individual plants (e.g., ten stem lengths per plant in 1981 versus three stems in 1980). There is a strong year-to-year consistency for most variables which is interpreted as support for last year's results and conclusions.

The same basic pattern follows from the Symphoricarpos oreophilus data (Table 32) as was observed for Amelanchier utahensis. In the 1981 growing season we made additional measurements on Symphoricarpos oreophilus to reflect other aspects of ecotypic differentiation. Branch length and branch angles were measured on garden plants that displayed different shapes. Varying both of these parameters can model different shrub shapes and differences in the way leaves are displayed to sunlight. A small 90° protractor was devised to measure the angles between leaf-bearing and support branches and between these branches and their higher order support branches. Branch lengths were measured and the data for leader and lateral branch lengths kept separate. For plants of the same population and even within a single plant the variances of these measurements were high; too high, in fact, to obtain an adequate sample.

Also in 1981, 40 individual Amelanchier utahensis plants flowered for the first time in the garden after being planted in 1978. Among the populations flowering no differences in the timing or amount of flowering were evident. But, since

Table 29. Sphaeralcea coccinea 1981 garden measurements. Results of one-way analysis of variance across seven populations for each measurement.

Garden Measurement		F Value†
Average phenological stage:	May 26	0.4
	Jun 3	2.4*
	Jun 11	0.9
	Jun 21	1.0
	Jul 1	2.5
	Jul 27	1.0
Average phenological stage after second flowering flush:	Jul 27	2.6*
Average number of flowers:	May 26	2.8*
	Jun 3	1.9
	Jun 11	3.0

†Significant F ratios are indicated: .05*, .01**, .001***.

Table 30. Purshia tridentata 1981 garden measurements. Results of one-way analysis of variance across four populations for each measurement.

Garden Measurement		F Value†
Average phenological stage:	May 20	0.6
	May 28	1.5
	Jun 5	0.9
	Jun 15	1.2
	Jun 24	0.6
	Jul 4	0.6
Plant height:	May 20	0.9
Canopy diameter:	May 20	0.1
Canopy area:	May 20	0.3

†Significant F ratios are indicated: .05*, .01**, .001***.

Table 31. Amelanchier utahensis 1981 garden measurements. Results of one-way analysis of variance across 25 populations for each measurement and comparisons with results from 1980 measurements.

Garden Measurement		F Value†	Significant in 1980
Average leaf			
Blade length:		14.6***	nm
Width		15.9***	yes
Petiole length:		5.1***	nm
Total length:		12.1***	yes
Length/width:		7.5***	yes
Terminal stem length:	Jun 3	6.3***	yes
	Jun 13	6.2***	yes
	Jul 21	3.1***	no
	Jul 31	2.9***	no
Lateral stem length:	Jun 3	7.7***	yes
	Jun 13	4.5***	yes
	Jul 21	2.4***	no
	Jul 31	2.4***	no
Stem growth rate:	Jun 3-Jun 13	2.1***	yes
	Jun 21-Jul 31	1.8*	yes
	Jun 3-Jul 31	3.2***	nm
Plant height:	Aug 4	1.8*	no

†Significant F ratios are indicated: .05*, .01**, .001***; "nm" means "not measured".

Table 32. Symphoricarpos oreophilus 1981 garden measurements. Results of one-way analysis of variance across 15 populations for each measurement and comparisons with results from 1980 measurements.

Garden Measurement		F Value†	Significant in 1980
Phenology:	May 30	3.3***	no
	Jun 9	2.9**	yes
Number of flowers:	May 30	3.9**	nm
Average leaf:			
Blade length		4.9***	nm
Width		1.8	no
Petiole length		6.4***	nm
Length		5.7***	no
Stem length:	Jun 4	3.2***	no
	Jun 14	3.4***	yes
	Jul 26	2.1*	yes
	Aug 4	2.2*	yes
Stem growth rate:	Jun 4-Jun 14	2.0*	yes
	Jul 26-Aug 4	2.0*	nm

†Significant F ratios are indicated: .05*, .01**, .001***; "nm" means "not measured".

differences in reproductive structures are often important, 10 fruits from each plant were collected to determine the number of locules and seeds per locule. No statistical differences were found among the populations, however. There were five locules per ovary and two seeds per locule in virtually every sample. For these traits and for phenological timing there were no significant differences among the populations that flowered.

However, all the flowering populations were collected from high elevations or from southern Wyoming, whereas the nonflowering populations were collected from lower elevations or from more southern sites. High elevation and northern sites have a shorter growing season. This suggests that short-season plants growing under long-season conditions (the common garden) may produce and accumulate more photosynthate. If these plants do not reproduce until reserves reach a threshold amount, this could have been accomplished sooner while growing through longer seasons.

To evaluate another aspect of adaptation we measured the water saturation deficit of leaves of A. utahensis and Symphoricarpos oreophilus. Two populations of Amelanchier utahensis were chosen, one from the moxt xeric and one from the most mesic original collection sites. Four Symphoricarpos oreophilus populations were chosen to represent the gradient of the original collection sites from xeric to mesic. Water saturation deficit did not differ significantly for Amelanchier utahensis but did for Symphoricarpos oreophilus. On a hot July afternoon, one population of S. oreophilus imbibed 30.7±4.4% of its total water content at saturation, while the others took up less (19.3±3.3, 20.5±3.7, and 25.7±0.8); this difference is significant at the .05 level. The population with the greatest amount of imbibition could therefore continue to take up water under even more xeric conditions where the others could not. This is evidence for physiological ecotypic adaptation since the common garden provides an essentially uniform environment. Furthermore, the ecotype exhibiting the greatest tolerance to xeric conditions was collected from the most xeric site.

Shrub Growth on Retorted Shale

The garden ecotype studies are directed mainly at adaptations that plants have to the climatic and edaphic regimes. The garden results allow speculation as to the ecotypes most likely to succeed under stress due to retorted shales in the soil profile, but no real test of this is possible in the garden. In order to obtain some predictive information for responses to shales in the soil substrate, four ecotypes of S. oreophilus thought likely to respond differently to the presence of retorted shale were propagated by stem cuttings. Likewise, two ecotypes of Ceratoides lanata were transplanted from the field and held over winter in Fort Collins. These and the plants grown from cuttings are being transplanted into the retorted shale successional plots at the Intensive Study Site. Six to ten individuals of each ecotype will be established in the native species-no fertilizer combinations on all profile treatments.

Plant responses in growth characteristics will be monitored over subsequent growing seasons. Since these plants will also be growing under competitive situations, this aspect of their response can be investigated as well. In general, the ecotypes which show growth responses typical of good competitors are expected to not be well adapted to the stress of retorted shale in the soil. On the other hand, the most stress adapted ecotypes should be inhibited where competition is greater.

In addition, 20-40 Symphoricarpos oreophilus cuttings have been propagated and provided to the Range Science Department for planting in the Revegetation Technique Plots. The study of shrub responses to retorted shale was also approached by measuring shrub growth on two sets of experimental plots already existing. These were the Retorted Shale Successional Plot at the Intensive Study Site (constructed in 1977) and plots established in 1973 by Berg and Harbert (1974) to study the vegetative stabilization of retorted shales in the Piceance Basin.

Height and two perpendicular canopy diameters were measured on 6-10 individuals of Ceratoides lanata and Atriplex canescens in selected treatments in the Retorted Shale Successional Plots at the Intensive Study Site. The treatments chosen included all three fertilizer application levels and all six soil-retorted shale treatments but only those plots that were sown with a mixture of native species. Of the shrubs found on these plots only Ceratoides lanata and Atriplex canescens occurred in sufficient numbers to sample (see progress report 1979 for plot design).

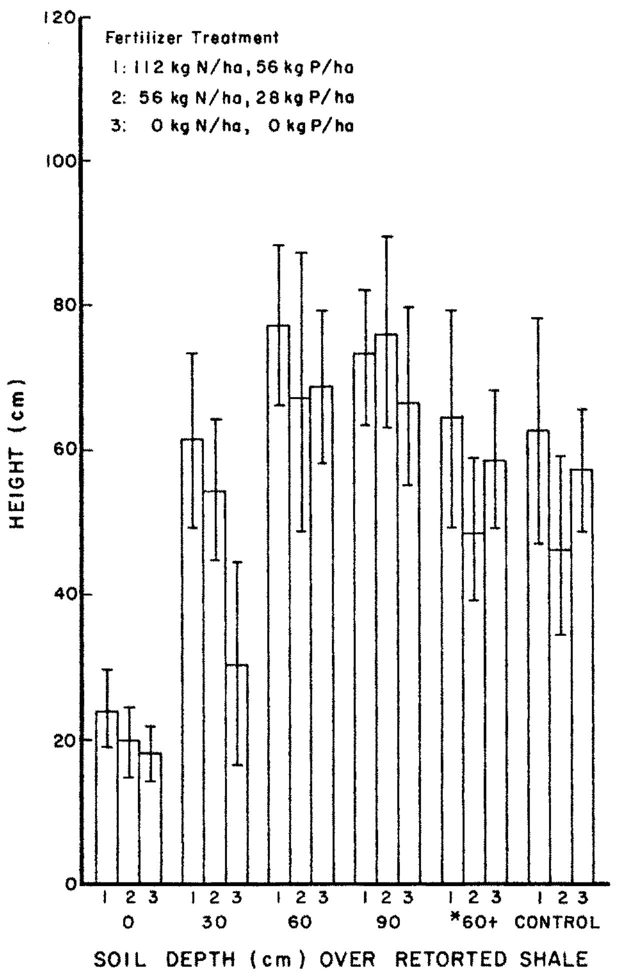

Fig. 39. Growth in height of Ceratoides lanata over retorted shale (*60+ = 60 cm topsoil over 30 cm rock capillary barrier.)

In both species the raw data for growth in canopy area were skewed toward large values. In order to reduce this skewness to allow for statistical testing, these data were subjected to a square root transformation which yields data on the square root of area, a term proportional to the radius of a circle having the same area as the vertical projection of the elliptic shrub canopy.

Both growth responses (height and canopy area) of Ceratoides lanata showed significant differences among retorted shale treatments within each fertilizer regime ($p<.01$). From Figs. 39 and 40, it is apparent that these differences are a result of depressed growth on both exposed retorted shale and that covered by 30 cm of soil. The effect is particularly marked in those plots given no fertilizer, and seems to be ameliorated somewhat by

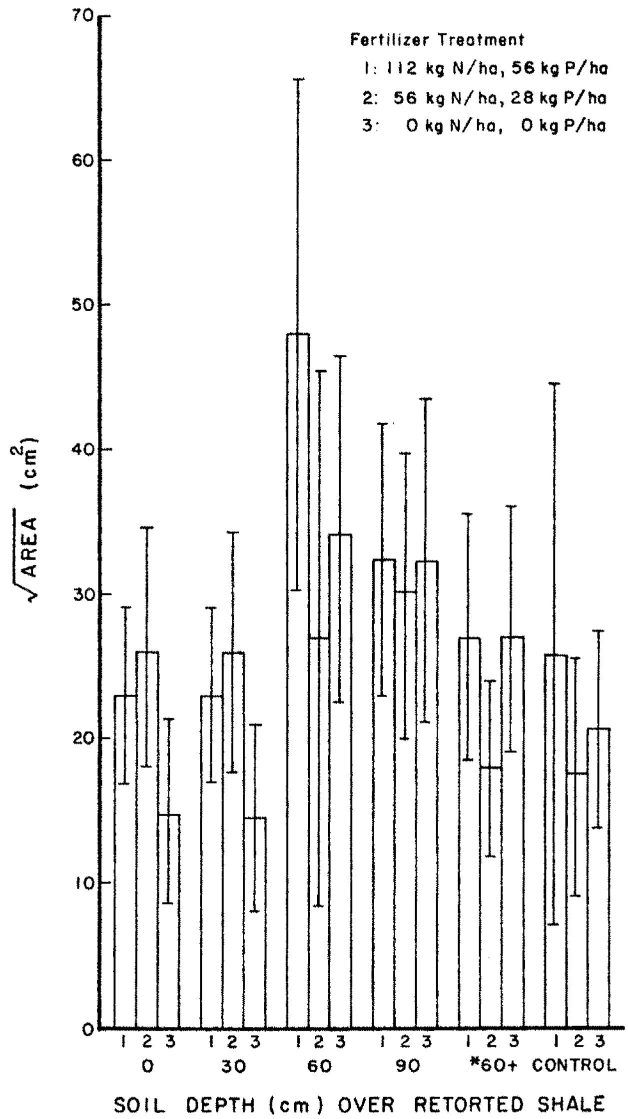

Fig. 40. Growth in canopy area (cm^2) of Ceratoides lanata over retorted shale (*60+ = 60 cm topsoil over 30 cm rock capillary barrier).

the application of fertilizer. Unless nutrient cycling is virtually complete in situ, it seems likely that future populations of shrubs, even on fertilized plots, will resemble present populations in unfertilized plots.

Plants of Atriplex canescens were sparse in the control plots and absent from plots with exposed retorted shale, although the species was included in the seed mixture sown on these plots. The absence of plants in the exposed retorted shale plots may be attributed to unfavorable conditions of soil chemistry, water relations, or temperature created by the dark color of the shale. Both height and canopy cover seem to be less in plots with 30 cm soil over retorted shale than in any other plots where Atriplex canescens was found (Figs. 41 and 42), but only plant height shows significant differences ($p < .05$).

Of the shrub species present on the vegetative stabilization plots, only Artemisia tridentata and Atriplex canescens were common enough to yield sufficiently large samples for growth measurements. Neither species showed any significant differences in height among treatments of USBM retorted shale (Table 33). A. canescens showed significant differences in height among treatments on TOSCO retorted shale on both north-facing and south-facing slopes, whereas Artemisia tridentata showed significant differences in height on north-facing TOSCO shale plots. In each case the growth of Atriplex canescens was depressed most on the retorted shale-to-surface plot and slightly less depressed on the plot having 15 cm soil over retorted shale compared to control plots. On north-facing plots over TOSCO retorted shale the growth of Artemisia tridentata was most depressed on the plot having 15 cm soil over retorted shale.

Harbert and Berg (1974) report resalinization of leached TOSCO shale without topsoil and of the plots in the treatment having 15 cm soil over TOSCO leached shale, but not in other treatments with greater topsoil cover. This resalinization is evidently caused by upward capillary movement of soil water carrying dissolved salts. The measured differences in growth correlate with these

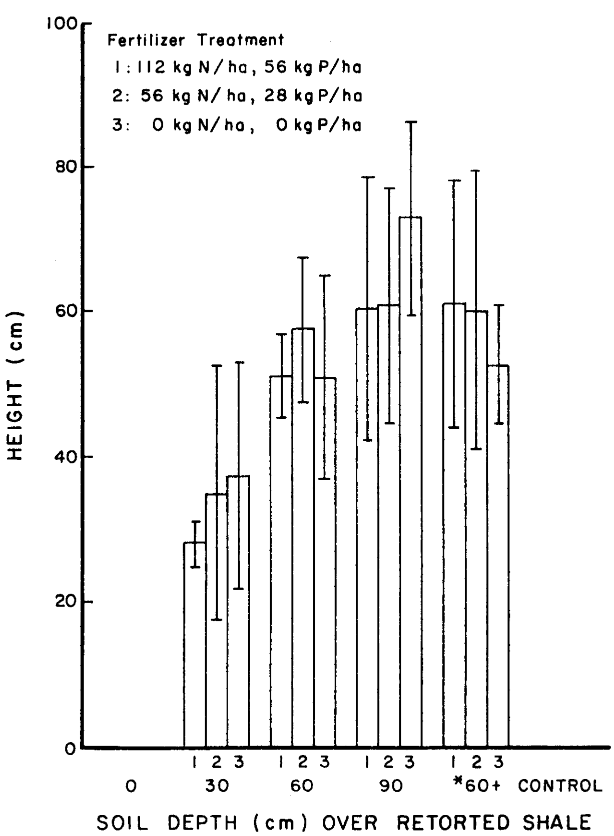

Fig. 41. Growth in height of Atriplex canescens over retorted shale (*60+ = 60 cm topsoil over 30 cm rock capillary barrier).

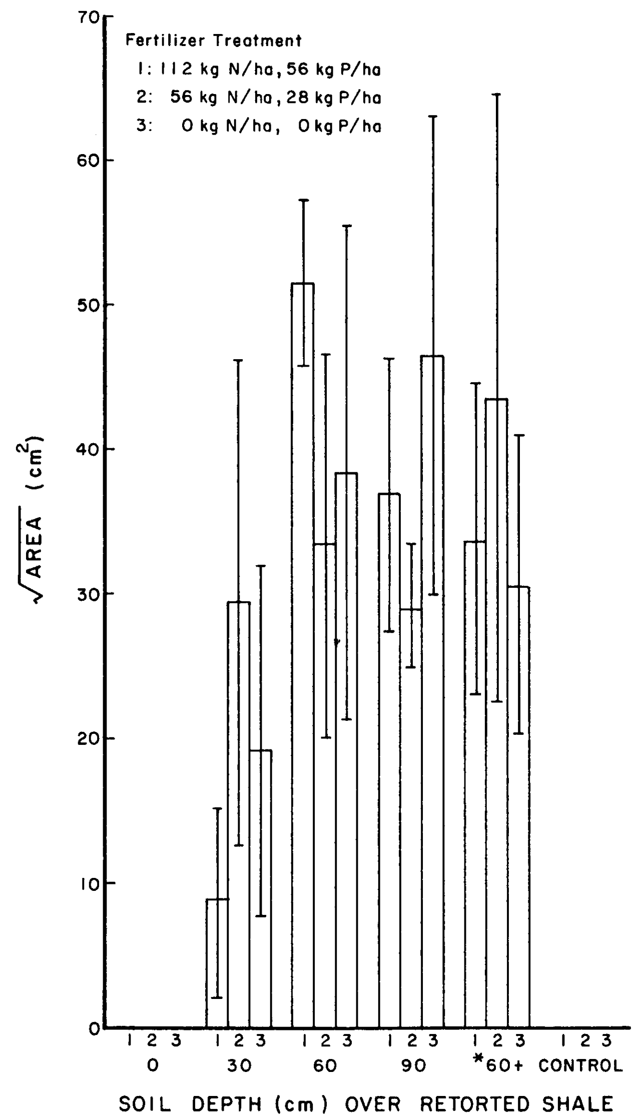

Fig. 42. Growth in canopy area (cm^2) of Atriplex canescens over retorted shale (*60+ = 60 cm topsoil over 30 cm rock capillary barrier).

Table 33. Growth in height of Artemisia tridentata and Atriplex canescens over TOSCO and USBM retorted shales. T0 = control, T1 = 30 cm soil over unleached shale, T2 = 15 cm soil over leached shale, T3 = leached shale to surface.

			T0		T1		T2		T3		
			Height	Number Plants	Height	Number Plants	Height	Number Plants	Height	Number Plants	F Value†
TOSCO	N-facing	Artemisia	62.0	4	54.5	4	34.75	6	54.17	6	3.40*
		Atriplex	43.3	10	42.4	10	31.89	9	28.6	10	7.91**
	S-facing	Artemisia	75.2	5	---	1	56.33	6	---	1	1.12
		Atriplex	33.6	10	81.9	10	32.4	10	31.37	8	83.11*
USBM	N-facing	Artemisia	60.6	10	70.33	9	86.9	11	---	3	2.67
		Atriplex	---	2	45.0	10	42.3	10	39.7	10	0.9
	S-facing	Artemisia	72.8	10	50.4	8	---	3	58.3	13	2.8
		Atriplex	47.0	10	48.9	10	40.0	10	38.4	10	1.5

†Significant F ratios are indicated: .05*, .01**.

observations (i.e., growth is reduced on treatments having TOSCO retorted shale to surface or 15 cm soil over TOSCO retorted shale). However, not all of these differences were statistically significant. Soil tests were made in 1981 to determine whether these soils were saline or if the 15-cm treatments have become desalinized. Electrical conductivity values were less than 2 mmhos/cm on soil over shale and in the range of 2-5 mmhos/cm in TOSCO retorted shale. These data indicate that the soil profile has not resalinized over time.

Although Purshia tridentata, rabbitbrush (Chrysothamnus spp.), Amelanchier utahensis, and Ceratoides lanata were seeded in these plots at the time of their construction, along with Artemisia tridentata and Atriplex canescens (Harbert and Berg 1974), they were not present in 1981, with the exception of a few Ceratoides lanata. During 1974 Artemisia tridentata seedlings had to be thinned in order to allow the growth of other plants (Harbert and Berg 1974). Since that time there has been some attrition in A. tridentata. In some plots only two or three individuals remain.

Thus, it appears that some environmental factor not related to retorted-shale treatments is constraining plant establishment on these plots. Only those shrubs naturally found in this landscape position in the Piceance Basin have proven successful on these plots. Other species seeded here did not survive, even on the control plots, despite intensive watering and fertilization during the first growing season after planting. Similar responses were observed on shrubs on the Retorted Shale Successional plot at the Intensive Study Site.

Field Extension of the Garden Studies--Work Initiated

The question of field confirmation of the significance of the garden ecotype results may be approached by making certain observations on naturally occurring populations along various stress gradients. To reiterate the essence of those results, the premise is that the ecotypes can reasonably be positioned along an abstract gradient of environments tracing from sites with high environmental stress to sites favorable for plant growth. Small, slow growing, nutrient conserving ecotypes are interpreted to be best suited for highly stressed environments; ecotypes with the opposite characteristics are best suited for low stress environments where competitive interactions become more important.

Predictions for which ecotypes are best suited to particular environments are thus tied to both stress and competition. An ecotype which does well in an environment of high stress is expected to do poorly where the environment is more favorable but competition is more intense. These predictions may be tested in two ways: first, by going to the various original ecotype collection sites and measuring competition and second, by measuring environmental features of the sties which relate directly to stress (e.g., low moisture availability, shallow soils, or short growing seasons).

Testing these predictions will permit the completion of another of the objectives, namely "to measure the responses of populations of a species directly in their native habitats and to interpret those responses with respect to ecotypic differentiation and reclamation suitability for specific conditions."

The problem, then, is to measure competition in natural situations. Preliminary work along this line was begun in 1981-1982 by modifying the testing methods from the literature for use in the Piceance Basin (Slauson et al. 1982). Because of its relative simplicity, the pinyon-juniper woodland was used for the development of appropriate methods.

There were some interesting results from this effort regarding ecological aspects of pinyon (Pinus edulis) and juniper (Juniperus osteosperma) of importance to reclamation. It appears that competition processes result in a structure of pinyon-juniper stands where mature individuals are regularly (uniformly) placed, even though juveniles are often aggregated (clumped). This suggests that for reclamation planning initial establishment of young trees in a fairly regular array may allow them to avoid some of the suppression, or even mortality, due to competition.

It was also evident that young trees of both species are almost exclusively found under larger trees or shrubs where considerable shade is provided. It has been reported (Meagher 1943 and Fowles 1965) that juveniles are not only shade tolerant but require shade for several years. Ideally, perhaps, young trees should be placed in the shade of a shrub nurse plant, which they could soon over top.

DISCUSSION

Results from garden plantings for the 1981 growing season substantially confirm the patterns found in 1980. There is no need, therefore, to repeat the discussion in last year's progress report. The salient points are that Oryzopsis hymenoides, Ceratoides lanata, Amelanchier utahensis, and Symphoricarpos oreophilus have elaborated ecotypes. The various ecotypes of these species are related in part to position along a gradient of stress to competition. Plants with genotypes producing small size and slow growth rates are adapted to the stress end; large, fast growing plants reflect an adaptation to competition. Plants of Purshia tridentata, Cercocarpus montanus, Atriplex canescens, Koeleria cristata, and Sphaeralcea coccinea have not expressed ecotypic differentiation, at least in the context of the populations within the Piceance Basin landscape.

That adaptations, when they occur, generally fit the simplified framework offered by the stress-to-competiton gradient is reinforced by some of the new studies undertaken this year. Plants growing under the stress of retorted shale tend to be smaller, indicating slower growth rates. Although the plants in these studies were not of different ecotypes, the fact that the phenotypes of the plants growing under different conditions vary in the way predicted from the stress-competition model suggests that selection pressure for these traits is in effect. Experiments where known ecotypes are planted into retorted shale plots will directly relate to this question.

Observations on the responses of shrubs to the presence of retorted shale in the soil profile suggest that some plants can indeed grow, although suppressed. How long these plants will persist can be told only by time. It is significant that the species found in sufficient numbers to sample were those species characteristic of the landscape surrounding the plots in question. Plants not ordinarily found in these areas, although seeded, did not survive or were maintained in very low numbers. Thus, the garden studies support findings of these field studies and the field studies support findings of the garden studies.

The field studies are aimed at identifying the climatic and normal edaphic constraints for plant growth within the overall environment. Given the concept of limiting factors (that the environmental factor which is below or above the tolerance range of the organism will limit growth no matter how favorable the other conditions) these results indicate that the presence of retorted shale in the soil profile may not be limiting. Climatic or ordinary edaphic conditions also constrain plant growth. Thus, in spite of the fact that on the vegetation stabilization oil shale plots several species were sown (irrigated, fertilized, and cultivated) the only shrub species abundant eight years later were species normally found in the landscape position of the plots (i.e., Artemisia tridentata and Atriplex canescens). Plants of Ceratoides lanata occurred in reduced amounts in the plots and also occur in this landscape in sparse amounts.

Studies initiated to investigate ecotype response to competition versus stress have not advanced far enough to yield substantive results for shrubs. However, intresting results concerning the ecology of Pinus edulis and Juniperus osteosperma relevant to reclamation were found. First, seedlings of these species are reasonably shade tolerant. In nature the majority of living juveniles of these species occur under the canopies of shrubs or other trees. If these species are seeded or transplanted as juveniles in reclamation, it would be important to place them in the shade of larger plants or some other shade. Second, in mature woodland stands older, larger individuals are distributed in a uniform manner. A reclamation practice of uniformly distributing young trees would avoid the delay of growth due to competition between young neighbors. In sum, plant these trees in shade and well separated.

SUMMARY AND CONCLUSIONS

Ecotypic differentiation is not strongly evidenced for the populations studied of Cercocarpus montanus, Purshia tridentata, Atriplex canescens, Koeleria cristata, and Sphaeralcea coccinea. Barring further evidence to the contrary, a range of source materials comparable to ours from Colorado can be used interchangeably for a variety of reclamation situations suitable to these species.

Ecotypic differentiation with respect to competitive ability, moisture, and short growing season stress has taken place for Symphoricarpos oreophilus, Amelanchier utahensis, Ceratoides lanata, and Oryzopsis hymenoides. Source materials should be selected with moisture and seasonal environments similar to the reclamation site to best ensure individual plant survival.

Ecotypic differentiation with respect to the timing and amount of phenological development also exists in Ceratoides lanata and Amelanchier utahensis with respect to the length of the growing season. Phenology of Symphoricarpos oreophilus is related to the radiation environment. Oryzopsis hymenoides also differs in phenology in a site specific way. Reclamation source material from sites comparable in these respects is recommended to best ensure long-term survival of the populations.

Shrubs planted in soil profiles containing retorted shale are smaller and slower growing. Further, only those species characteristic of the environments of the specific site have been successful.

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NITROGEN SUPPLY BY LEGUMES AND OTHER FERTILITY SOURCES FOR DISTURBED LANDS

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OBJECTIVES

The overall objective of this subproject is to determine the long-term fertility requirements and methods of meeting these requirements on N- and P-deficient soil materials used as plant growth medium for reclamation of land disturbed by oil shale development in northwestern Colorado.

A. Examine contribution and potential of the legume-Rhozobium nitrogen-fixing system:

1. Determine nitrogen status in the soil, the legume top growth, and the top growth of associated grasses, as influenced by species, soil medium, and initial fertilizer application.
2. Determine by acetylene-reduction assay the relative rates of nitrogen-fixation ability of selected legumes in the same plot measured for "1".
3. Analyze the growth, seed setting ability, and seasonal N-fixation ability of several species of native and introduced legumes, as to their suitability in rehabilitation.
4. Compare nodule formation on legume roots growing in surface disturbed topsoil with those growing in or near subsurface retorted oil shale, wherever such plots can be destructively sampled.

B. Determine how much nitrogen has been lost from the zone of incorporation and moved into the retorted shale below the topsoil due to leaching.

METHODS

Legume Plots

These plots were established in the summer and fall of 1978 at the Intensive Study Site in a randomized block design with 14 test items (species or mixtures), 3 levels of fertility, 2 soils, and 3 replications (Figs. 43 and 44). The study area was constructed by first removing the vegetation from the site. The topsoil and subsoil were then removed and stockpiled separately. The area was excavated to a total depth of 60 cm and the grade leveled from east to west. Soil was replaced so that each replication contained both a subsoil and a topsoil replacement in a random order. A 5-m border was left between each soil treatment to insure a homogeneous soil type within the plot.

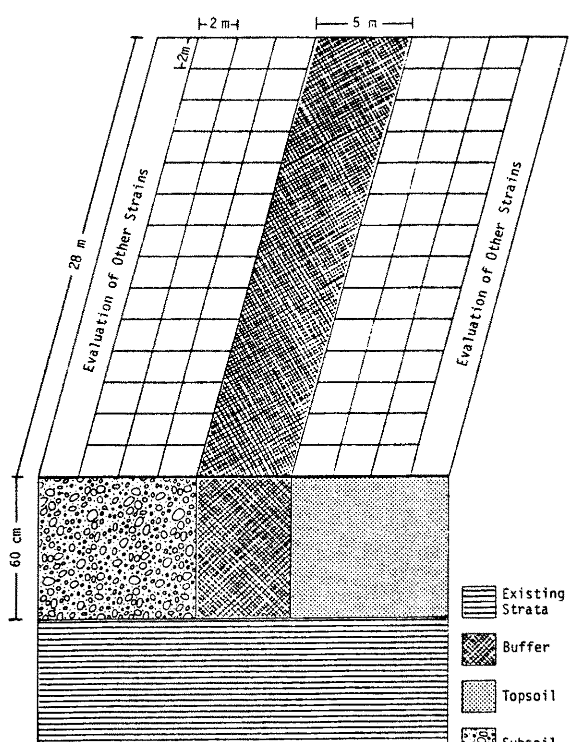
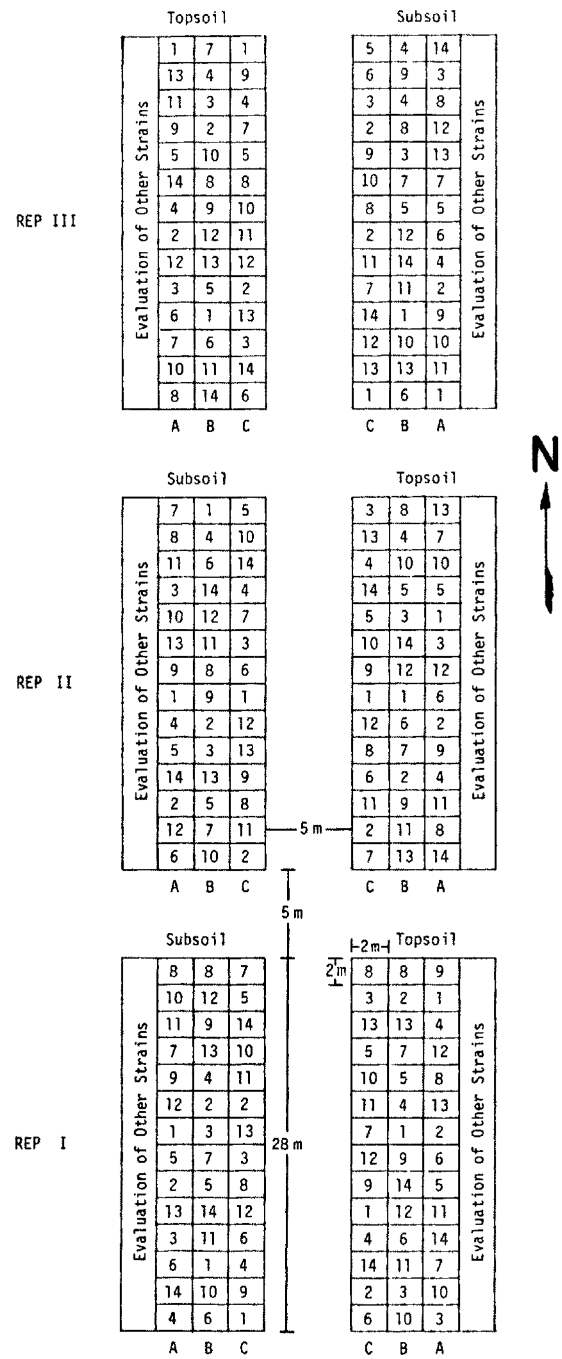


Fig. 43. Profile configuration of one replication (REP I) from the Legume Study.



Species Seeded

1 = Sweetvetch
2 = Sweetvetch plus inoculum
3 = Penngift crownvetch
4 = Lutana cicer milkvetch
5 = Eski sainfoin
6 = Ladak alfalfa
7 = Sweetvetch and grass
8 = Sweetvetch plus inoculum and grass
9 = Penngift crownvetch and grass
10 = Lutana cicer milkvetch and grass
11 = Eski sainfoin and grass
12 = Ladak alfalfa and grass
13 = grass
14 = grass

Fertilizer Rate

A = 0 kg N/ha
B = 56 kg N/ha
C = 112 kg N/ha

Fig. 44. Field map of the Legume Plot.

After construction was complete, the soil was sampled in each panel to a depth of 30 cm with a soil probe. To correct the phosphorus deficiency, the plot was fertilized at 190 kg P/ha with triple superphosphate in September 1978 just prior to seeding. Nitrogen and phosphorus fertilizer was applied with a cyclone-type spreader and disked in. This high level of phosphorus should prevent P deficiency symptoms in the legumes throughout the duration of this study.

The plot was seeded (October 14-15, 1978) approximately six weeks after fertilization and seedbed preparation to allow the fluffy soil to settle. Table 34 lists the species planted, the seed source, and the inoculum treatment. The legumes were broadcast seeded at a rate of 430 seeds/m^2 on a lightly raked seedbed. Inoculum was mixed with a small quantity of soil and sprinkled over the subplots. The area was again lightly raked to cover the seeds and distribute the inoculum. The legume-grass mixtures (Table 35) were seeded at the same density, divided equally between legumes and grasses. Portable wooden frames with dimensions of each subplot (2x2x0.3 m) were placed around each subplot during seeding as a guide to seed placement.

Along the outside panels of each replicate several additional strains of each legume, plus lupine seed collected near the Intensive Site, were row seeded (Table 36). Each of the 90 plots consisted of a pair of 2-m rows, 1 m apart. These species have been studied for adaptation to the Piceance Basin area: see previous progress report (Cuany et al. 1981, pp. 79-89.). Roots have also been examined for degree of nodulation in conjunction with acetylene reduction assays.

Table 34. Species planted, seed source, and type of inoculum used on the Legume Plot (broadcast seeded October 14-15, 1978).

Treatment	Species Seeded and Source	Inoculum
1	Hedysarum boreale utahensis Sweetvetch, Stewart and Sons	None
2	Hedysarum boreale utahenis Sweetvetch, Stewart and Sons	Native soil
3	Coronilla varia Penngift crownvetch, Northrup King	Commercial
4	Astragalus cicer Lutana cicer milkvetch, Northrup King	Commercial
5	Onybrychis viciifolia Eski sainfoin, Northrup King	Commercial
6	Medicago sativa Ladak alfalfa, Northrup King	Commercial
7	Treatment 1 plus native grass mixture†	None
8	Treatment 1 plus native grass mixture	Native soil
9	Treatment 3 plus native grass mixture	Commercial
10	Treatment 4 plus native grass mixture	Commercial
11	Treatment 5 plus native grass mixture	Commercial
12	Treatment 6 plus native grass mixture	Commercial
13	Native grass mixture	None
14	Native grass mixture	None

†Refer to Table 35 for a list of grasses in the native grass mixture.

Table 35. List of native grasses used in grass-legume seed mixture.

Common Name	Scientific Name
1. Rosana western wheatgrass	Agropyron smithii
2. Sodar streambank wheatgrass	Agropyron riparium
3. Bearded bluebunch wheatgrass	Agropyron spicatum
4. Indian ricegrass	Oryzopsis hymenoides
5. Green needlegrass	Stipa viridula
6. Durar hard fescue	Festuca ovina duriuscula
7. Sherman big bluegrass	Poa ampla

Table 36. Legumes and seed sources in adaptation and N_2-fixation study.

Species	Major Plots (broadcast)	Minor Strains (in rows)
Sweetvetch	Stewart's†	Stewart's, ISS, E/ISS, Pipeline
Alfalfa	Ladak	Ladak
Cicer milkvetch	Lutana	Lutana, C-4, HFY, Monarch, Sugarbeet-2, Wellington, 20-15,
Crownvetch	Penngift	Penngift, Emerald, Chemung
Sainfoin	Eski	Eski, Melrose, Remont
Lupine	---	031, 142, 225, 232
Purple prairieclover	---	Kaneb
Swainsons pea	---	San Luis Valley

†With and without local soil for rhizobia inoculum. All others were preinoculated with the most appropriate inoculum and seeded October 15, 1978.

Plant and Soil Analyses

Aboveground plant materials were sampled in the summer of 1979, 1980, and 1981 and analyzed for total nitrogen. Soils were sampled annually and analyzed for total N, ammonium, and nitrate nitrogen to determine the effect of legumes on nitrogen content of the soil. Methods used were from Bremner (1965).

Soils were sampled in early spring (April 1981) in snowy weather. Ten samples were taken to a depth of 15 cm and composited. The status of N in the soil and effective nitrogen uptake should be reflected in the N content of the grasses and legumes. Forage quality and digestibility can be directly related to the N content of that forage (Baylor 1974). Plant material sampling was done by taking random leafy stems and blades without completely destroying the whole plants in the summer of 1981 in an attempt to reduce the adverse impact on plant growth and legume survival. All grasses were harvested on June 8, 1981. Sainfoin, alfalfa, and crownvetch were sampled on June 26, 1981; the remaining legumes were harvested July 25 in order to sample similar physiological stages.

Other plant data were gathered using two quadrats randomly placed by separate teams on each quadrat on July 1-3, 1981. Density, biomass, and cover values were determined as an average of these quadrats in each plot. Viewed together these analyses of soil and plant nitrogen coupled with the other plant data provided the basis for evaluation of this study.

The data from 1981 were analyzed with SPSS multivariate analysis of variance program MANOVA. Single degree of freedom contrasts were used to compare the effect of the various seed mixtures.

Acetylene Reduction Assays

On various dates in 1981 plants in the broadcast seeded plots (Objective 2) and the row seeded legume strain plots (Objective 3) were sampled destructively, using the east half of the broadcast plot or the north row of the paired rows. The procedure used was similar to that used by Klein et al. (1981) except that plant root systems were not soaked in water and standard incubation times were longer. Plants were dug with a soil core 25 cm diameter and 25 cm depth. The soil was shaken and separated gently from the roots; the tops were severed at ground level. The root system with all attached and detached nodules was put into a 980-ml widemouthed Mason jar, sealed with a lid which had a 1.5 cm diameter hole plugged with a standard rubber septum. One-tenth of the gaseous atmosphere (90 ml) was withdrawn and replaced by injecting 90 ml of acid-scrubbed acetylene. The jars were shaded from direct sunlight and incubated in the field for two hours.

Two kinds of controls were employed: (1) The ethylene blanks which consisted of a root system of each species incubated in a jar with no acetylene injected; this would allow detection of endogenous ethylene production by roots or nodules (no ethylene was detected in any of the blanks). (2) The acetylene blank which had no roots but the standard amount of acetylene was injected; this served as a check on the use of the acetylene amount as an internal standard in all jars and for detecting any ethylene contaminant in the acetylene supply (none was detected).

After the incubation, a gas sample was drawn in to a 10-ml evacuated tube and the tubes were transported to the laboratory at Fort Collins for analysis of acetylene and ethylene amounts using gas chromatography. Root systems, including nodules, were placed in a cooler for later separation and oven drying to determine dry weights of roots and nodules for each plant analyzed. Milligrams of nodule per gram of root were calculated.

Two greenhouse studies of acetylene reduction were done: the first on seedlings of four species of legume (the same as tested from the row plots) and the second on progenies of 16 single plants of sweetvetch with associated checks. The former test was grown for eight weeks in separate pots (six replications) of topsoil and subsoil from the Piceance Basin, watered daily to field capacity. Five seeds were germinated and planted into each pot, being simultaneously inoculated by sprinkling peat-based Rhizobium prepared by the Nitragin Company. The seedlings were collected at eight weeks of age and incubated in jars as before, but for two and four hours incubation in the 10% acetylene atmosphere. Other procedures of gas chromatography and root and nodule weighing were standard.

The latter greenhouse test on seedling progenies used 10 seedlings per pot which contained fertile topsoil (Fort Collins clay loam) mixed 1:1 with fine sand. Inoculation was as above, on the four replications planted, which were thinned to five seedlings per pot at three weeks. Greenhouse temperatures were maintained at 24/16°C day/night, and two 500 watt lights were on daily from 6 a.m. to 8 p.m. for supplemental illumination. At eight weeks of age the acetylene reduction assay was done as before and nodule, root, and shoot dry weights were obtained.

Long-Term Fertility Study

The Long-Term Fertility Study was established on the Intensive Study Site in the summer and fall of 1977 to determine the fertility needs and methods of meeting these needs for nitrogen- and phosphorus-deficient soils. The topsoil study was constructed with 60 cm of topsoil material that was mixed thoroughly and placed over compacted Paraho retorted shale.

In each study three replicates were established with 19 treatment plots and one control plot per replicate. The treatments included levels of annually applied nitrogen (N) over a four-year period, single applications of N equivalent to four-year annual application, wood waste with N, sewage sludge, and N and phosphorus (P) combined as a single application when the plots were initially drill seeded (Tables 37 and 38).

Rodent and lagomorph damage was extensive on the topsoil study during the winter of 1978-1979. The plot was, therefore, reseeded in May 1979. The seedbed was prepared using a chisel plow with two alternate rows of chisels 20 cm apart. The area was chiseled once perpendicular to the original seed rows. All plots were broadcast seeded with a native seed mix (Table 37). The seed was broadcasted and covered by dragging a weighted chain-link fence over the plots. Approximately 15 cm of water were applied over six weeks during the spring

Table 37. Species mixture and seeding rates used on the Long-Term Fertility Plots.

Common Name	Scientific Name	Seeding Rate PLS (hg/ha)
1. Rosana western wheatgrass	Agropyron smithii	1.1
2. Sodar streambank wheatgrass	Agropyron riparium	1.1
3. Bearded bluebunch wheatgrass	Agropyron spicatum	1.1
4. Indian ricegrass	Oryzopsis hymenoides	1.1
5. Green needlegrass	Stipa viridula	1.1
6. Durar hard fescue	Festuca ovina duriscula	0.6
7. Shermans big bluegrass	Poa ampla	1.1
8. Alkali sacaton	Sporobolus airoides	0.6
9. Globemallow	Sphaeralcea munroana	0.6
10. Sweetvetch	Hedysarum boreale	1.1
11. Palmer penstemon	Penstemon palmeri	0.6
12. Stansbury cliffrose	Cowania mexicana stansburiana	2.2
13. Green ephedra	Ephedra viridis	1.1
14. Fourwing saltbush	Atriplex canescens	1.1
15. Winterfat	Ceratoides lanata	1.1
16. Antelope bitterbrush	Purshia tridentata	1.1

and summer of 1979 to insure uniform germination and emergence of the planted seed.

Soil samples were obtained in the spring of 1978, 1979, and 1980 from these topsoil fertility plots. In 1978 the soil was sampled using an Oakfield probe 2 cm in diameter. Twenty cores 30 cm deep were obtained at random locations within each treatment plot. These cores were composited and air-dried in preparation for further analysis.

In 1979 and 1980 the composite samples consisted of 10 cores per treatment plot as described above. The Topsoil Over Retorted Shale Study was sampled at two depths, 0-30 cm and 30-60 cm. The sampling depth stopped at the soil-retorted shale interface. The underlying shale was not sampled.

The soil samples were analyzed each year for electrical conductivity, pH, NH_4^+-N, NO_3^--N, total nitrogen, and extractable phosphorus. Soils were air-dried and ground to pass through a 2-mm sieve prior to analysis.

The decision was made that no plant or soil samples would be taken or measurements made on these plots in 1981 with the exception of depth samples down through the retorted shale on plot Treatments 3, 7, 11, 15, and 20 as noted in Table 38. Treatments 3, 7, 11, and 15 were chosen

Table 38. Fertilizer treatments for the five substudies of the Topsoil Fertility Study.

Treatment	Fertilizer Treatment
	Annual Rates of N†
1	56 kg N/ha applied annually for a four-year period
2	112 kg N/ha applied annually for a four-year period
3‡	224 kg N/ha applied annually for a four-year period
4	448 kg N/ha applied annually for a four-year period
	Single Rates of N
5	224 kg N/ha in a single application
6	448 kg N/ha in a single application
7‡	896 kg N/ha in a single application
8	1,792 kg N/ha in a single application
	Single Rates of N as Above Plus Wood Wastes (ww)
9	224 kg N/ha plus 11,120 kg ww/ha
10	448 kg N/ha plus 22,400 kg ww/ha
11‡	896 kg N/ha plus 44,800 kg ww/ha
12	1,792 kg N/ha plus 89,700 kg ww/ha
	Sewage Sludge (ss) Plus ww
13	56,000 kg ss/ha
14	112,000 kg ss/ha plus 22,400 kg ww/ha
15‡	224,000 kg ss/ha plus 44,800 kg ww/ha
	P§ and N Interaction
16	896 kg N/ha plus 56 kg P/ha in a single application
17	112 kg N/ha plus 192 kg P/ha in a single application
18	896 kg N/ha plus 192 kg P/ha in a single application
19	112 kg N/ha plus 56 kg P/ha in a single application
	Control
20‡	130 kg P/ha

†N applied as NH_4NO_3.

‡Plots sampled to 122-cm depth in 1981.

§P applied as triple superphosphate.

because of the relatively high N addition. If NO_3 were going to be leached into the retorted shale and beyond from any of the plots, it surely would from these high N treatments.

The treatments were an annual application of 224 kg N/ha for a four-year period (Treatment 3) and an initial application of 896 kg N/ha (Treatment 7) which was identical to the total nitrogen added in four years in Treatment 3. Treatments 11 and 15 were similar in that each had 44.8 metric tons of wood wastes applied to immobilize inorganic nitrogen for further mineralization. The difference between these treatments was that 11 had the same 896 kg N/ha as was applied to Treatments 3 and 7, but applied with wood wastes. Treatment 15 used an application of 224 metric tons/ha of anaerobically digested sewage sludge from Glenwood Springs, Colorado. The control was Treatment 20, with no application of nitrogen. All of these treatments except Treatment 15 received an application of 130 kg P/ha in the form of superphosphate to eliminate phosphorus as a nutrient limiting to plant growth. Inorganic nitrogen was applied in the form of ammonium nitrate.

Samples of soil were taken in triplicate with a king tube at nine depth intervals as defined in Table 39. Data were analyzed by analysis of variance. Least significant difference test was used at the 0.05 level of probability to identify significantly different means. Two factors of the design were analyzed as a split-plot with treatment factor as the main plot and the depth as the split-plot within each treatment.

RESULTS TO DATE

Legume Plots

Fertility maintenance on disturbed lands depends on adequate and timely supply of nitrogen, phosphorus, and other nutrients either as fertilizers or through enhanced activity of elements of the ecosystem. Their relative usefulness in promoting the growth of appropriate erosion-controlling vegetation is being sought through comparing the contributions of legumes with rhizobia and mycorrhiza to the results of long-term fertility applications, i.e., biological and chemical pathways.

Table 39. Description of sampling depths with symbol designations for each treatment identified in Table 38.

Symbol	Soil Sample Location	Depth From Surface (cm)
G	Grab	Surface
T	Topsoil	5-15
S	Subsoil	30-45
A	Above 1st interface	50-61
B	Below 1st interface	61-70
SH	Shale	80-90
AA	Above shale interface	100-107
BB	Below shale interface	107-115
R	Undisturbed soil	>122

Nitrogen fixation by the legumes symbiotic with *Rhizobium*, in plots established in 1978, needs continuing study to evaluate nitrogen contribution to the ecosystem through direct and indirect transfer as exhibited in the N status of the soil and in the aboveground biomass of legumes and grasses.

The hypothesis is that nitrogen can be transferred to associated grasses after the legume plant begins to add nitrogen-rich, easily mineralized organic matter to the soil. Nitrogen availability is regarded as mainly controlled by N cycling within live plants (Woodmansee 1979). Since legumes have the demonstrated ability to fix atmospheric nitrogen and introduce this N into the internal mineral cycle of the soil-plant systems, certain introduced and native legumes are evaluated in this study. The role of legumes in reclamation has been stressed by McKell (1975), especially as suppliers of N to grasses and other forbs.

Grasses are commonly grown in association with legumes as forage because data consistently show that higher yields as well as better seasonal distribution of forage can be obtained when compared with N-fertilized grasses (Baylor 1974). Grass establishment is critical to revegetation because grasses aid in soil development, prevent erosion, intercept percolation water that could contaminate groundwater, and provide nutritious forage for wildlife and livestock.

Application of inorganic N can supply this nutrient immediately, but substantial losses have commonly been noted. Since 99.5% of the N in a shortgrass prairie is organically bound (Woodmansee et al. 1978), establishing long-term maintenance levels with inorganic N can be difficult. A large pool of inorganic N suppresses legume nodulation and subsequent nitrogen fixation. Nitrogen fertilization has also been found to promote the establishment of aggressive volunteer annuals which outcompete both planted species and plants more advanced successionally than these annuals.

The ability of legumes to provide much of their own N requirement through symbiosis and the transfer of nitrogen to associated grasses with high N demands, justifies the use of legume-grass associations in various situations including vegetation stabilization. This transfer brings atmospheric N into the internal N cycle of the disturbed soil-plant system. Microbially-controlled mineralization of several N-rich substrates supplies available nitrogen for uptake. Significant amounts of N can be made available to associated grasses by root excretions of the actively growing legume (Vincent 1965). The principal pathway of internal mineral cycling is the shedding of leaf and root materials, adding N-rich organic matter to the soil (Sosebee 1977). The turnover of these materials could vary from 2-5 years for aboveground litter to 20 years for roots. However, young and unsuberized roots decompose in a matter of weeks. These roots can provide substantial organic matter in a disturbed soil because of the recurring sloughing of these roots due to fluctuating conditions. The grasses can form a dense mat of roots where the entire root zone functions as a rhizosphere. Thus, the entire root zone can store nitrogen within organic materials, living and dead. Once nitrogen is stored by the plant, the nutrient is protected against leaching and denitrification. This study provided a basis for determining the value of planting legumes in association with grasses for vegetative stabilization in the Piceance Basin.

Plant Measurements and Soil and Plant Nitrogen Levels on Broadcast Plots

Total Biomass Measurements

Table 40 shows the plant biomass as affected by species, species mixtures, fertilizer application rate, topsoil, and subsoil. The data indicate that only 6 of 14 species and species mixtures (3, 6, 8, 11, 13, and 14) increased in biomass with increasing fertilizer application. The remaining species and mixture either decrease or do not follow a regular pattern in response to additional applications of fertilizer. In every case (except 4 and 9) the highest fertilizer treatment caused a greater biomass production than the control. Even though there is not an overwhelming biomass response to initial fertilizer N, it appears to be evident even with some of the legumes and certainly with the grasses.

The plant biomass is consistently greater on the topsoil plots than the subsoil plots except for seed mixtures, sweetvetch (inoculum), sweetvetch (inoculum) + grasses, and alfalfa + grasses. Even though there was a response to fertilizer, it did not overcome the effect of topsoil compared to the subsoil.

Plant biomass was greater in the pure grass plots than the pure legume plots with the grass-legume plots producing an intermediate amount compared to the grasses or legumes alone. Among the legumes, alfalfa produced the greatest biomass while crownvetch produced the least.

Total Density Measurements

Fertilizer applications caused a consistent increase in plant number in all plots for all seed mixture treatments except for Species 8, 11, 12, and 13 as shown in the data in Table 41. Even with these treatments, the highest fertilizer rate caused greater plant density than the control.

There was a general tendency for density to be greater on the topsoil plots than the subsoil plots

Table 40. Mean plant biomass values (g/0.25 m^2) as affected by species, species mixture, fertilizer treatment, subsoil, and topsoil.

		Species													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Soil	Fertilizer Application Rate (kg N/ha)	Sweetvetch	Sweetvetch (inoculum)	Crownvetch	Cicer milkvetch	Sainfoin	Alfalfa	Sweetvetch + Grasses	Sweetvetch (inoculum) + Grasses	Crownvetch + Grasses	Cicer milkvetch + Grasses	Sainfoin + Grasses	Alfalfa + Grasses	Grasses	Grasses
Topsoil	0	44.7	35.6	26.1	49.0	38.0	55.8	72.7	42.0	72.4	61.5	57.9	65.3	79.6	64.2
Topsoil	56	53.3	58.7	35.9	50.8	40.0	71.6	50.4	57.5	76.7	58.2	51.3	73.3	95.3	52.4
Topsoil	112	39.8	47.8	32.8	62.8	64.5	88.0	64.3	65.3	74.4	86.8	58.7	57.1	93.9	88.2
Subsoil	0	35.1	36.2	31.2	58.9	33.8	67.6	55.0	66.4	60.7	47.4	40.6	56.7	68.2	54.0
Subsoil	56	40.4	55.0	27.4	38.8	26.9	52.4	46.4	51.3	47.5	47.3	53.8	52.6	61.8	61.3
Subsoil	112	35.7	52.4	33.3	45.1	44.2	80.8	68.1	62.2	63.4	62.5	60.8	103.2	68.2	92.4
Topsoil	---	48.6	47.4	31.6	54.2	47.5	71.6	62.5	55.0	71.9	68.9	56.0	60.8	89.6	74.2
Subsoil	---	38.2	48.3	30.7	47.9	32.6	66.9	56.5	60.0	57.2	52.4	51.8	70.8	66.1	69.2
Subsoil + Topsoil	0	39.9	35.9	28.7	54.0	38.8	61.7	63.9	54.2	66.5	54.5	44.1	61.0	73.9	59.2
Subsoil + Topsoil	56	46.9	56.8	31.6	44.8	33.5	61.8	48.4	54.4	62.9	52.8	52.6	52.1	78.6	65.8
Subsoil + Topsoil	112	43.0	50.4	33.0	38.7	54.3	84.4	66.3	63.8	60.8	74.7	59.8	82.1	81.1	90.2

Table 41. Mean plant density values (#/0.25 m^2) as affected by species, species mixture, fertilizer treatment, subsoil, and topsoil.

Soil	Fertilizer Application Rate (kg N/ha)	Species 1 Sweetvetch	2 Sweetvetch (inoculum)	3 Crownvetch	4 Cicer milkvetch	5 Sainfoin	6 Alfalfa	7 Sweetvetch + Grasses	8 Sweetvetch (inoculum) + Grasses	9 Crownvetch + Grasses	10 Cicer milkvetch + Grasses	11 Sainfoin + Grasses	12 Alfalfa + Grasses	13 Grasses	14 Grasses
Topsoil	0	68.5	60.7	35.8	49.2	16.8	12.0	19.2	32.0	9.7	12.0	13.7	13.2	9.8	10.2
Topsoil	56	46.5	77.8	45.8	53.0	15.8	13.3	23.5	32.8	8.2	12.8	10.5	12.7	7.0	11.8
Topsoil	112	98.2	110.2	44.2	61.2	23.5	17.7	53.5	45.0	24.0	13.0	14.3	21.5	8.5	9.5
Subsoil	0	43.2	47.8	34.0	25.3	11.7	12.0	13.8	16.7	11.3	9.3	14.2	9.0	7.2	8.3
Subsoil	56	72.0	59.0	37.0	44.5	23.2	12.8	17.8	11.5	28.2	9.0	13.3	9.3	5.7	7.7
Subsoil	112	94.7	75.0	55.2	48.5	34.5	10.0	16.5	25.5	26.0	23.0	15.5	8.3	8.3	11.8
Mean Topsoil	---	69.5	82.9	41.9	54.4	19.3	13.9	32.0	36.6	14.4	12.6	11.9	15.9	8.4	10.5
Mean Subsoil	---	69.9	62.0	42.0	34.0	23.1	11.8	15.5	17.9	21.8	13.8	14.9	8.9	7.5	9.3
Subsoil + Topsoil	0	55.8	54.7	34.9	37.2	14.2	12.0	16.5	24.6	11.2	10.7	13.9	11.3	6.8	9.8
Subsoil + Topsoil	56	59.2	68.4	41.4	48.8	19.5	13.1	20.7	22.2	18.2	10.9	11.9	11.0	6.3	9.8
Subsoil + Topsoil	112	96.3	90.1	49.7	56.1	25.7	13.8	35.0	35.2	25.0	18.0	14.9	14.9	8.4	10.7

although Treatments 1, 3, 5, 9, 10, and 11 were exceptions as shown in the data for Mean Subsoil in Table 41. The mean plant densities of grasses were significantly lower than the legumes even though the biomass was generally greater. The plant densities of the legume-grass mixtures were again intermediate compared to the grasses alone or legumes alone. The density of sweetvetch was greatest while alfalfa was least. Because of the rather large size of the plants and the high biomass production of alfalfa, the growth habit differences and/or greater biomass per plant account for the apparent difference in space occupied per plant for the two species. Similar reasoning may account for the high biomass of grasses but comparatively low density.

Total Plant Cover

In keeping with the above pattern there was a general tendency for fertilizer to cause an increase in plant cover though the differences were not as great as they were in the biomass and density data (Table 42). Additionally, the plant cover on the topsoil plots tended to be greater than the subsoil plots, but the differences were not satistically significant. There were no discernible differences in total plant cover when comparing grasses and legumes.

Nitrogen Content in Legumes and Grasses

Seed mixture Treatment 13 (grasses) in Table 43 indicates a gradual and consistent increase in percent N in grasses with increases in nitrogen fertilizer application. These differences, however, are generally not great enough to be statistically significant. Similar increases occurred with the grasses mixed with legumes with the exceptions of topsoil for seed mixture Treatments 7, 8, and 11 and subsoil for seed mixture Treatments 7 and 12. This inconsistency of increase in percent N in the herbage as fertilizer application increased probably indicates that the fertilizer treatments are not greatly influencing percent N in the grasses grown with the legumes. Over two and one-half years have elapsed since the fertilizer N was applied.

It is of interest to note that the percent N in the grasses grown with legumes (Treatments 7, 8, 9, 10, 11, and 12) on topsoil was not increased as much compared to the percent N in grasses alone (13) as was the case in the subsoil plots. Apparently the topsoil plots were not markedly deficient in N or the grasses grown with legumes on the subsoil plots obtained some nitrogen from the N fixation of the legumes.

Table 44 shows the mean percent N in grasses and in legumes from combined topsoil and subsoil

Table 42. Mean plant cover values (%) as affected by species, species mixture, fertilizer treatment, subsoil, and topsoil.

		Species													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Soil	Fertilizer Application Rate (kg N/ha)	Sweetvetch	Sweetvetch (inoculum)	Crownvetch	Cicer milkvetch	Sainfoin	Alfalfa	Sweetvetch + Grasses	Sweetvetch (inoculum) + Grasses	Crownvetch + Grasses	Cicer milkvetch + Grasses	Sainfoin + Grasses	Alfalfa + Grasses	Grasses	Grasses
Topsoil	0	15.4	11.8	11.6	14.6	12.0	12.6	18.6	13.7	17.8	15.1	16.0	16.1	18.4	14.7
Topsoil	56	17.4	19.5	12.5	14.9	11.5	18.4	14.3	15.1	15.7	11.8	11.4	19.5	17.8	17.4
Topsoil	112	16.3	22.8	12.6	20.7	16.2	18.4	18.9	18.4	14.3	17.4	12.5	14.0	16.4	19.4
Subsoil	0	12.5	12.9	12.3	16.6	8.1	13.3	14.1	12.0	11.4	12.7	11.2	13.2	16.5	13.2
Subsoil	56	13.4	14.8	9.5	13.6	20.8	12.3	14.4	13.1	13.1	11.4	13.9	13.0	13.4	14.2
Subsoil	112	17.0	16.2	14.4	14.4	14.9	18.9	16.9	15.3	15.7	15.2	14.5	20.6	15.5	19.2
Mean Topsoil	---	16.4	18.0	12.2	16.7	13.2	16.5	17.3	15.7	15.9	14.8	13.3	16.5	17.5	17.2
Mean Subsoil	---	14.3	14.6	12.1	12.4	12.7	14.8	15.1	13.5	18.2	13.1	13.2	15.6	15.1	15.5
Subsoil + Topsoil	0	8.8	12.3	12.0	15.6	10.0	13.8	16.3	14.1	14.6	13.9	14.6	14.7	17.5	14.0
Subsoil + Topsoil	56	15.4	13.9	11.0	14.3	9.9	15.4	14.3	14.1	14.3	13.4	12.7	16.2	15.6	15.8
Subsoil + Topsoil	112	16.7	19.0	14.7	19.5	14.3	18.6	17.0	16.9	15.0	16.3	13.5	17.3	16.8	19.3

Table 43. Mean nitrogen content (%) of grasses as affected by species, species mixture, fertilizer treatment, subsoil, and topsoil.

		Species						
		7	8	9	10	11	12	13
Soil	Fertilizer Application Rate (kg N/ha)	Sweetvetch + Grass	Sweetvetch (inoculum) + Grasses	Crownvetch + Grasses	Cicer milkvetch + Grasses	Sainfoin + Grasses	Alfalfa + Grasses	Grasses
Topsoil	0	1.08	1.33	1.10	1.11	1.04	0.89	1.16
Topsoil	56	1.19	1.38	1.18	1.32	1.16	1.26	1.18
Topsoil	112	1.13	1.19	1.29	1.66	1.13	1.33	1.41
Subsoil	0	0.93	1.03	0.99	0.97	0.73	1.15	0.88
Subsoil	56	1.02	1.06	1.20	1.04	0.93	0.83	0.93
Subsoil	112	0.96	1.23	1.34	1.37	0.96	1.25	0.99

plots in an attempt to evaluate the effect that legumes have on the percent N of the grasses grown with the legumes and the effect that grasses have on the percent N of the legumes grown with the grasses. As expected, after studying the data in Table 44, Treatments 9, 10, and 13 showed a consistent increase in the percent N of the grasses with increasing fertilizer application rates. Additionally, Treatment 12 showed a similar pattern even though the pattern of percent N increase for that treatment in Table 43 in the subsoil plots was somewhat different. When the data for the topsoil and subsoil plots were combined, the influence of the data for the topsoil plots were sufficiently great to overcome the nonconsistent increase in the percent N with fertilizer application in the subsoil plots.

The percent N in the legumes generally increased as the N application increased. Only in Treatments 5, 6, and 8 are the percent N values no greater where 112 kg N/ha were added than that of the control (0 kg N/ha). A comparison of the percent N of the legumes grown alone (Treatments 1, 2, 3, 4, 5, and 6) with the percent of the legumes grown with grasses (Treatments 8, 9, 10, 11, and 12) shows no consistent pattern of increase or decrease in percent N with increased nitrogen application. It therefore is not possible to

Table 44. Mean plant nitrogen (%) as affected by species, species mixture, fertilizer treatment, subsoil, and topsoil.

Means for Combined Subsoil and Topsoil	Fertilizer Application Rate (kg N/ha)	Species 1 Sweetvetch	2 Sweetvetch (inoculum)	3 Crownvetch	4 Cicer milkvetch	5 Sainfoin	6 Alfalfa	7 Sweetvetch + Grasses	8 Sweetvetch (inoculum) + Grasses	9 Crownvetch + Grasses	10 Cicer milkvetch + Grasses	11 Sainfoin + Grasses	12 Alfalfa + Grasses	13 Grasses
Grass	0	---	---	---	---	---	---	1.00	1.21	1.04	1.04	0.89	1.02	1.02
Legume	0	2.07	1.89	2.04	2.81	1.84	2.03	1.95	3.0	1.92	2.34	1.80	2.03	---
Grass	56	---	---	---	---	---	---	1.10	1.22	1.18	1.18	1.04	1.04	1.05
Legume	56	2.25	2.44	1.98	2.81	1.86	1.96	2.50	2.38	2.14	2.89	1.86	2.19	---
Grass	112	---	---	---	---	---	---	1.04	1.20	1.32	1.55	1.04	1.30	1.20
Legume	112	2.30	2.49	2.25	3.26	1.77	2.03	4.06	2.41	2.08	3.41	1.93	2.11	---

Table 45. Mean soil nitrogen (ppm) content as affected by species, species mixture, fertilizer treatment, subsoil and topsoil.

Soil	Fertilizer Application Rate (kg N/ha)	N Form	Species 1 Sweetvetch	2 Sweetvetch (inoculum)	3 Crownvetch	4 Cicer milkvetch	5 Sainfoin	6 Alfalfa	7 Sweetvetch + Grasses	8 Sweetvetch (inoculum) + Grasses	9 Crownvetch + Grasses	10 Cicer milkvetch + Grasses	11 Sainfoin + Grasses	12 Alfalfa + Grasses	13 Grasses
Topsoil	0	NO_3	3.74	5.35	4.02	4.11	3.24	4.19	4.04	4.33	4.24	4.15	5.04	3.71	2.99
		NH_4	116.0	120.0	135.17	116.5	94.5	95.83	75.5	103.5	90.0	114.5	124.0	109.67	136.5
		Total	1326	1395	1283	1361	1326	1309	1179	1213	1274	1213	1256	1309	1369
Topsoil	56	NO_3	7.06	9.21	9.13	6.52	12.50	7.72	10.06	7.63	11.57	11.32	18.97	26.34	8.07
		NH_4	96.0	102.83	122.0	129.0	89.5	116.5	121.0	111.5	125.0	69.3	93.3	103.0	126.5
		Total	1300	1257	1387	1265	1231	1430	1309	1291	1343	1517	1266	1213	1317
Topsoil	112	NO_3	10.82	9.41	10.74	9.04	8.40	9.25	7.30	7.83	11.35	6.72	6.27	6.61	9.97
		NH_4	130.5	109.5	137.3	76.5	138.7	105.5	97.5	71.5	27.1	118.0	108.8	126.0	117.5
		Total	1499	1326	1361	1264	1430	1326	1413	1274	1161	1451	1395	1343	1361
Subsoil	0	NO_3	2.93	2.04	3.59	2.91	2.03	4.77	2.78	4.13	7.91	5.54	2.82	2.79	3.15
		NH_4	69.0	67.0	61.5	52.8	77.5	60.5	97.5	56.6	77.0	85.0	73.0	74.0	80.0
		Total	1013	1369	1007	1439	1170	1007	1127	1170	1023	1127	1210	962	1057
Subsoil	56	NO_3	3.72	5.37	5.37	6.33	5.60	6.82	9.18	5.73	4.19	6.74	5.18	4.16	4.7
		NH_4	62.3	65.5	58.0	52.0	65.8	76.5	52.5	49.0	81.5	64.5	55.5	71.5	49.0
		Total	893	962	901	945	1109	1105	893	849	1031	936	945	1031	884
Subsoil	112	NO_3	9.07	10.23	7.14	8.26	6.68	8.45	11.12	5.69	8.86	10.00	8.99	7.97	7.50
		NH_4	53.5	65.0	82.0	69.5	67.5	66.7	37.5	58.0	76.5	75.5	51.5	52.3	60.5
		Total	962	988	1005	1019	1023	1027	979	1005	1031	988	1005	962	937
Subsoil + Topsoil	0	NO_3	3.34	3.70	3.81	3.51	2.64	4.48	3.41	4.23	6.08	4.85	3.93	3.25	3.07
		NH_4	92.5	93.5	98.34	84.65	86.0	78.17	86.5	80.0	83.5	99.75	98.5	91.84	108.25
		Total	1169	1382	1145	1400	1248	1158	1153	1191.5	1149	1170	1233	1136	1213
Subsoil + Topsoil	56	NO_3	5.39	7.29	7.25	6.43	9.05	7.27	9.62	6.68	7.88	9.03	12.08	15.25	6.89
		NH_4	79.15	84.17	90.0	90.5	77.65	96.5	86.75	80.25	103.25	66.9	74.4	87.25	87.75
		Total	1097	1110	1144	1105	1170	1268	1101	1070	1187	1227	1106	1122	1101
Subsoil + Topsoil	112	NO_3	9.95	9.82	8.94	8.65	7.54	8.85	9.21	6.76	10.11	8.36	7.63	7.29	8.74
		NH_4	92.0	87.25	109.65	73.0	103.1	86.1	67.5	64.75	51.8	96.75	80.15	89.17	89.0
		Total	1231	1157	1183	1142	1227	1177	1053	1088	1027	1058	1108	962	997

conclude that legumes grown alone will take up more N than when legumes are grown with a grass mixture. This is in contrast to the data for the grasses and is expected from theoretical considerations.

Soil Nitrogen in Broadcast Plots

The data in Table 45 indicates that the NO_3 in the soil generally increased with increases in fertilizer application. Even though the increase is not consistent for seed mixture Treatments 5, 10, 11, and 12, there is no case where the control (0 kg N/ha) plots had as much NO_3 as the high fertilizer treatment (112 kg N/ha).

There is no consistent pattern discernible for the NH_4 or total N data nor were there any differences sufficiently great to be statistically significant.

Effect of Native Soil Inoculum on Sweetvetch

Sweetvetch, the only native legume used on the broadcast plots, was evaluated with and without the application of native soil inoculum. The comparison was made by placing soil taken from the proximity of the native sweetvetch plants and mixing it with the plots with Treatments 2 and 8. The treatments without inoculum were 1 and 7. Plant biomass, density, and cover (as well as soil nitrogen) were not affected by the inoculum. The nitrogen content of the sweetvetch plants showed an interesting effect, however. With no nitrogen applied (Fig. 45) Treatment 8 plants showed a nitrogen content of 3.0% (composite of topsoil and subsoil). The same species in Treatment 7 without this inoculation had a nitrogen content in the sweetvetch of only 1.95%. Apparently the inoculum has had an effect on nitrogen uptake by sweetvetch when no nitrogen was added; when 56 and 112 kg N/ha were added, a similar pattern was not evident. The extra nitrogen negated the need for nitrogen fixation. This is a commonly observed pattern in soils.

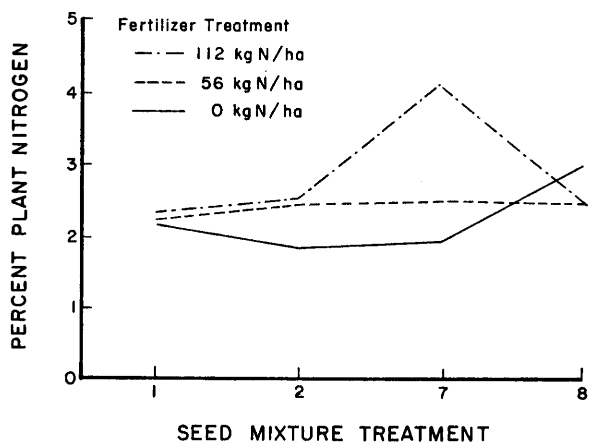


Fig. 45. Nitrogen content of sweetvetch planted with (7,8) and without (1,2) grasses and with (2,8) and without (1,7) inoculum (subsoil and topsoil are pooled).

Acetylene Reduction Assays on Broadcast Plots

In examining the contribution and potential of the legume-Rhizobium nitrogen-fixing system, the second objective was to determine, by acetylene reduction assay, the relative nitrogen-fixing potential of selected legumes in the same plot as previously discussed. Assays were made in June 1980 by Klein et al. (1981), but they revealed no significant differences in acetylene reduction among the treatments sampled. However, a significant increase in nodulation was found at 0 kg N/ha fertilizer compared with 56 kg N/ha which produced 20 mg nodules per gram of root versus only 11 mg per gram, respectively. This was believed to be due to the depressing effect of higher available N on the rhizobial infection and nodulation process of the legumes.

On July 12, 1981 the legumes in the broadcast plot study were evaluated for acetylene reduction rates as a means of estimating their current N_2 fixation potential.

Three replicates of each species, on each of two soil types and three initial N fertilizer levels, were assayed for a total of 108 samples. The species were: alfalfa, cicer milkvetch, sainfoin, sweetvetch, sweetvetch (inoculum), and sweetvetch (inoculum) + grass. The results showed that only 13 plants produced measurable amounts of ethylene, out of the 108 sampled. Only these 12% were actively reducing acetylene, and it was not possible to conduct a valid statistical analysis.

There was no obvious relationship with soil type or nitrogen level, or even among species of legume. Only one alfalfa plant out of 18 was active (i.e., reducing acetylene to ethylene) although 15 out of 18 had nodules. One sweetvetch plant produced ethylene although the roots contained no detectable nodules; this was either endogenous ethylene (despite the zero values from ethylene blanks), or there were Rhizobia in stem segments that did not appear like nodules. In either case, it would be impossible to calculate specific nodule activity (SNA) as μmols ethylene evolved $\cdot$ g^{-1} nodule $\cdot$ $hour^{-1}$.

The depressing effect of fertilizer N (in fall 1978) on the nodulation process may have been dissipated by 1981 because there were 8.6, 12.7, and 4.4 mg of nodules per gram of root at the 0, 56, and 112 kg N/ha rates, respectively. Six out of 36 plants had no nodules at the zero N rate, and 10 and 13 out of 36 at the two higher N rates, respectively, had no nodules which may suggest some slight inhibition remaining. Even plants with nodules were showing only sporadic and erratic evidence of nitrogenase activity in July 1981.

The probable cause for the low number of plants actively reducing acetylene is the time of year at which the sampling was conducted. By the middle of July, the plants had been exposed to moisture stress for a few weeks and senescence was beginning; senescence is frequently accompanied by nodule loss and a decrease in rate of nitrogen fixation (Hardy et al. 1973). Perhaps if the analysis had been conducted in May or early June, a higher percentage of the plants would have been active.

Assays of Row Plots and Greenhouse Comparisons for Nitrogen Fixation

The third of the legume/nitrogen fixation objectives is to study seasonal N-fixation ability, rate of growth, and seed-setting ability of several species of native and introduced legumes relative to their suitability in rehabilitation. The first of these topics is addressed here, and the other two in a later section. As stated earlier, the row plots for this study were established in October 1978 on replaced topsoil and subsoil. For each species two plots were hand-drilled on each of the soil types; a plot consisted of two 2-m rows, 1 m apart. The north row of each pair was devoted to destructive sampling, with two plants dug on each of three sampling dates.

Observations were made on four species of legumes out of the eight legume species in the separate row plots. Three of the legumes, alfalfa (Medicago sativa L.), sainfoin (Onobrychis viciifolia Scop.), and cicer milkvetch (Astragalus cicer L.), were species introduced to the Piceance Basin along with a fourth, sweetvetch (Hedysarum boreale Nutt.), which is a native to the area (Table 46). The acetylene reduction assay was employed three times during the summer of 1981 (May 25, July 11, and August 18) to estimate nitrogen fixation potentials. Additionally, milligrams of nodules per gram of root were calculated for each plant analyzed in the assay in order to observe trends in the amount of nodulation over the growing season.

Results indicate that more plants were evolving ethylene from acetylene on both soils in the May analysis (Table 47) than in either the July or August evaluations (Table 48) and that acetylene reduction rates expressed as SNA were highest on this analysis date. With the exception of Hedysarum, the legumes had higher acetylene reduction rates on topsoil than on subsoil for the May analysis. Astragalus strains had the highest SNA on topsoil (32.6 and 24.2 μmols), and Onobrychis strains had the lowest (4.4 and 5.7 μmols). For both the July and August analyses only five of the 56 plants analyzed on each date produced measurable amounts of ethylene. SNA was much lower for both the latter dates as compared with the May analysis; values ranged from 0.005 to 0.30 μmols for July and from 0.05 to 4.41 μmols for August. The amount of moisture received at the Piceance Basin over the 1981 growing season no doubt influenced date comparisons of this field study. More moisture was available to the plants in May than in July or August, and SNA rates were highest in May.

A related greenhouse study conducted in June and July of 1981 in Fort Collins utilized seedlings of the same species and strains of legumes (Table 46) as in the Piceance Basin study, with the addition of one alfalfa strain. For this analysis gas samples were collected after two and four hours of incubation; the rates of ethylene production per hour worked out to be the same.

No statistically significant differences in SNA were observed either between strains within species or between soil types (Table 49). Statistical differences were, however, apparent between species. The Medicago strains had an average SNA rate of 115.8 μmols which was significantly higher than the other three species, and the Hedysarum strain average of 88.4 μmols was significantly higher than the Onobrychis and Astragalus which had averages of 22.4 μmols and 21.4 μmols, respectively. The greenhouse SNA rates were higher than those observed in the field study. This may be due in part to the fact that the greenhouse plants were inoculated eight weeks before they were analyzed and were still seedlings, whereas the field plants were inoculated two and one-half years before they were evaluated. Additionally, the greenhouse plants received water daily, while the field plants were never irrigated.

Comparisons of milligrams of nodules per gram of root for each species in the field study (Table 50) indicated that Astragalus, Medicago, and Onobrychis generally decreased over the summer of 1981 (at least on the dates sampled) while Hedysarum increased from May to July and decreased from July to August. The May-July increase and subsequent decrease were significant at $\alpha = 0.05$ only for the ISS strain of sweetvetch.

Table 46. Species, strains and seed sources of plant materials used in row plot and greenhouse studies.

Species	Strain	Source
Astragalus cicer L.	Monarch	Dr. C. Townsend USDA-ARS Fort Collins, CO
	HFY (High Forage Yield)	Dr. C. Townsend USDA-ARS Fort Collins, CO
Hedysarum boreale Nutt.	ISS	Piceance Basin, CO
	Stewarts	W. R. Stewart & Sons Ephraim, UT
Medicago sativa L.	Ladak	Northrup King Co.
	Saranac†	Northrup King Co.
Onobrychis viciifolia Scop.	Eski	Northrup King Co.
	Remont	Northrup King Co.

†Used in greenhouse study only.

Table 47. Number of plants active and rate of activity in legumes analyzed at the Piceance Basin, Colorado, May 25, 1981. Soil temperature: 12°C, this approximates the temperature of the incubation jars.

		Number of Plants Active†		Average μmols C_2H_4 Evolved · g^{-1} Nodule · hr^{-1}			
		Subsoil	Topsoil	Subsoil	S.E.	Topsoil	S.E.
Astragalus	HFY	3/4	2/4	9.8	4.6	32.6	31.8
	Monarch	2/4	2/4	14.1	8.2	24.2	19.7
Hedysarum	ISS	2/4	3/4	15.4	9.5	13.3	5.4
	Stewarts	3/4	2/4	6.9	3.5	4.9	4.5
Medicago	Ladak	2/4	2.4	8.9	7.4	10.7	7.0
Onobrychis	Eski	4/4	4/4	2.6	0.7	4.4	1.4
	Remont	3/4	4/4	3.3	2.2	5.7	1.9

†Refers to the number of plants of total analyzed, actively reducing acetylene (e.g., 3/4 means 3 plants active out of 4 analyzed).

Table 48. Number of plants active and rate of activity in legumes analyzed at the Piceance Basin, Colorado. Soil temperature: July 11, 1981--16.5°C; August 18, 1981--18.0°C. These temperatures approximate the temperatures of the incubation jars on each analysis date.

		July 11, 1981		August 18, 1981	
		Number of Plants Active†	Average µmols C_2H_4 evolved · g^{-1} Nodule · $hour^{-1}$	Number of Plants Active†	Average µmols C_2H_4 evolved · g^{-1} Nodule · $hour^{-1}$
Astragalus	HFY	1/8 s‡	0.11	1/8 s	0.7
	Monarch	1/8 s	0.10	1/8 s	4.41
Hedysarum	ISS	0/8	---	1/8 s	2.65
	Stewarts	0/8	---	---	---
Medicago	Ladak	0/8	---	1/8 s	2.58
Onobrychis	Eski	2/8 s,t	0.005 0.30	1/8 t	0.05
	Remont	1/8 t	0.02	0/8	---

†Refers to the number of plants of total analyzed, actively reducing acetylene (e.g., 1/8 means 1 plant active out of 8 analyzed).

‡s = subsoil; t = topsoil.

Table 49. Average activity of plants in June 1981 greenhouse study. Numbers followed by the same letter within a column are not significantly different at $\alpha = 0.05$ by Duncan's Multiple Range Test.

		Average µmols C_2H_4 evolved · g^{-1} nodule · $hour^{-1}$ (topsoil and subsoil combined)			
		2-Hour Incubation	Species Average	4-Hour Incuabtion	Species Average
Astragalus	HFY	17.5	21.4 a	27.4	24.5 a
	Monarch	23.3		21.6	
Hedysarum	ISS	88.3	88.4 b	71.9	75.7b
	Stewarts	93.5		79.5	
Medicago	Ladak	123.0	115.8 c	141.7	134.0 c
	Saranac	108.6		126.3	
Onobrychis	Eski	19.8	22.4 a	28.9	25.8 a
	Remont	25.0		22.7	
		S.E. = 12.2		S.E. = 10.6	

Table 50. Nodule weight to root weight comparison of legumes analyzed over the 1981 growing season at the Piceance Basin, Colorado. Numbers followed by the same letter within rows and columns are not significantly different at $\alpha = 0.05$ by Duncan's Multiple Range Test.

		Average mg Nodule · g^{-1} Root (dry weight; topsoil and subsoil combined)				
		May 25	Jul 11	Aug 18	Mean	S.E.
Astragalus	HFY	2.3 a	3.4 a	1.4 a	2.4	0.7
	Monarch	4.3 a	3.3 a	0.5 a	2.7	1.9
Hedysarum	ISS	2.2 a	7.0 b	0.6 a	3.3	1.1
	Stewarts	2.7 a	4.9 a	0.6 a	2.7	1.3
Medicago	Ladak	1.2 a	1.1 a	0.2 a	0.8	0.4
Onobrychis	Eski	158.4 c	89.4 c	45.3 c	97.7	56.2
	Remont	80.3 c	81.1 c	17.9 c	59.8	26.1

The greenhouse plants (Table 51) had higher weights of nodules per gram of root than the field plants, and statistical differences were observed in the greenhouse between the two Onobrychis strains and between Onobrychis and the other three species. These are, of course, seedling plants and also were analyzed within a few weeks after being inoculated, whereas the field plants were over two years old when analyzed. In July these field plants were showing senescent foliage and other indications of moisture stress. The evident scarcity of reinoculation in the field, combined with a lack of photosynthate from the leaves for nodule maintenance and activity, may explain the low nodule to root ratio and also the low acetylene reduction activity at later dates.

Based on the results of this research the question arises: Should these legumes be used for revegetation in the Piceance Basin area? The

Table 51. Nodule weight to root weight comparison of legumes analyzed in the greenhouse on July 31, 1981. Numbers followed by the same letter are not significantly different at $\alpha = 0.05$ by Duncan's Multiple Range Test.

		Average mg Nodule · g^{-1} Root (dry weight; topsoil and subsoil combined)
Astragalus	HFY	79.3 a
	Monarch	60.3 a
Hedysarum	ISS	34.2 a
	Stewarts	45.0 a
Medicago	Ladak	39.4 a
	Saranac	46.2 a
Onobrychis	Eski	108.8 b
	Remont	151.1 c

acetylene reduction rates of the field-tested legumes and their nodulation patterns in late May, July, and August indicate that the plants may not have been self-sufficient at those dates in obtaining their own needs for reduced nitrogen through the symbiosis with Rhizobium. Thus, from these data alone it would be hard to justify the use of these plants for providing extra nitrogen for associated grasses. However, if other criteria such as biomass production and physical appearance of these plants were examined, they would provide valuable diversity which is necessary to the landscape and current regulations. The plants appeared to be green, healthy, and vegetatively vigorous during both the 1980 and 1981 spring seasons and part way into the summers, although seed production was consistently low. Additionally, in the pure stand and grass mixture study both Onobrychis and Medicago did especially well. Analysis of the acetylene reduction rates of the legumes in that study (July 1981) indicated a similarly low activity compared to the legumes in the row study.

It is advisable to consider characteristics such as top growth, competition, seedling vigor (a measure of photosynthate production necessary to support rhizobial growth), and total nitrogen content before deciding whether or not to use such legumes for revegetation purposes. In addition, the effects of mycorrhizal fungi, as discussed by Redente and Reeves (1981), should be considered. They observed a significant increase in acetylene reduction rates of seedlings inoculated with Glomus (a vesicular-arbuscular mycorrhiza) and Rhizobium as compared to those inoculated with Rhizobium only. The nitrogen level of the aboveground plant material was found to be slightly higher in those plants inoculated with both symbionts as compared to the plants inoculated only with Rhizobium. Perhaps a selection program for genetic improvement of heritable traits (such as Rhizobium infection and acetylene reduction) similar to that of Duhigg et al. (1978) and Seetin and Barnes (1977) with alfalfa would help create legumes better able to fix atmospheric N_2.

Growth and Seed-Setting Ability of Legumes

Another part of the third objective is the analysis of growth and seed-setting ability of legumes in row plots, discussed in previous annual reports as well as in the preceeding section. Observations have continued on the eight species of introduced and native legumes. As before, crownvetch (Coronilla varia L.) is less vigorous than alfalfa , sainfoin, or cicer milkvetch. The two native legumes Lupinus argenteus Pursh and Petalostemon purpureum (Vent.) Rydb. appear of marginal value at the Intensive Study Site. Both crownvetch and Swainsons pea (Sphaerophysa salsula (Pall.) DC) are now spreading by rhizomes, the former to 0.5 m and the latter up to 4 m from the mother plants.

There are two locations where growth and seed production are being evaluated, one being the Intensive Study Site row plots where flowering of the four more important species had barely started at the beginning of June 1981 but resulted in practically no seed set in July except for a few seedpods on sainfoin. There was much evidence of pod abortion, and moisture was evidently limiting. At the second location the best two native legumes, sweetvetch and lupines are being evaluated in more detail for seed-setting in nursery plots in Meeker (Environmental Plant Center) and Fort Collins (Rigden and Stroh Farms).

Estimates of individual plant seed production and the variability among plants are important to future expanded utilization of these species for reclamation. They also enable propagation of the best possible strain to produce seed and plants for testing on industrial sites. Seed production from the nurseries is summarized in Table 52. Seed yield has increased over the three years (1979-1981) at the Rigden Farm. The average yield of 14 consistently high yielding plants in 1981 was 21.62 g clean seed per plant, a yield which on field scale may exceed 200 kg/ha. There has also been a slight increase in weight per 100 seeds. The 14 high yielding progenies have been bulked for multiplication as an experimental strain. In addition, these 14 selected progenies were included in a greenhouse test for germination, seedling growth and acetylene reduction, described below.

Out of a new nursery (Stroh) established in Fort Collins with plants mainly from the Stewart seed source in Utah and derived through bulk harvest from the Rigden plot in Fort Collins, only 46 plants out of 192 yielded harvestable seed. These plants yielded from 0.03 to 6.99 g clean seed per plant. Three of them were selected for inclusion in the greenhouse experiment for growth and acetylene reduction assay.

The multiplication rows at Meeker yielded poorly in 1981, which leaves some doubt in propagation of enough plants of Piceance Basin origin to test this strain in 1982 in the industrial sites. For all plants of the Intensive Study Site (ISS), east of ISS, and Pipeline accessions total seed yield was only 4.0 g which would be scarcely enough to get 300 seedlings for both nursery and test plot work. Further cooperative work with the Environmental Plant Center staff at Meeker is necessary to derive the most benefit from their collection of northwest Colorado strains as well as the seed production aspects.

A thesis by Stephen Kenny (1981) described several traits of lupines important for reclamation, such as seeding growth, acetylene reduction as a measure of nitrogen fixation potential, and alkaloid content. Lupine seed production remains a restriction in utilization of this species for reclamation. For this reason, concentration has been made on sweetvetch as more applicable to the main 1800-2400 m zone in the Piceance Basin. Nevertheless, it should be possible to develop a strain of lupines with high N_2-fixation, low alkaloids, and vigor appropriate to the 2200-2900 m zone on the ridges of the Basin.

Sweetvetch Progeny Test for Seedling Growth and N_2 Fixation Potential

The sweetvetch progeny test was conducted to measure the acetylene reduction rates of progeny of plants selected from the Fort Collins nurseries for

Table 52. Seed yields and seed weights (100-seed weight) for the sweetvetch nurseries.

Nursery	Year	Plants Present	Plants w/Seed	Average Seed Yield (g)	Range in Seed Yield (g)	Average 100-seed Weight (g)	Range in 100-Seed Weight (g)
Rigden 1977†	1979	102	11	8.56	1.6 -21.2	---	---
	1980	88	57	5.13	0.15-33.03	0.82	0.59-1.15
	1981	88	77	7.95	0.36-33.10	0.90	0.62-1.33
		Selections‡	14	21.62	10.22-33.10	0.95	0.68-1.33
Stroh 1980	1981	192	46	1.43	0.03- 6.99	1.09	0.82-1.43
		Selections‡	3	6.61	6.19- 6.99	1.17	1.02-1.26
Meeker 1979	1980	32§	---	none			
	1981	29	22	0.25¶		0.80	

†Flowered in 1978, but no seed was set. In 1979 only 11 best plants were harvested.

‡Plants selected for high seed yield in this nursery (e.g., 14 out of 77 and 3 out of 46).

§Short rows about 3 m each, estimate 8 plants per row.

¶Total yield of 22 rows was only 5.47 g of seed, most of this from the Pipeline and Stewarts accessions.

high seed yield. The ultimate goal of this and other work with sweetvetch is to produce a synthetic variety with high nitrogen fixing potential (among other useful characteristics) for revegetation purposes.

In November 1981 the seeds of 16 progenies and one collection were germinated in the laboratory. Germination tests showed 2-26% germination after seven days, without scarification; upon scarifying the hard seed which had not germinated, another 67-90% germinated within three days. So, the total viability was 86-100%. Seed collected from Intensive Study Site and east of there in July 1978 still showed 86% viability.

Data collected for the greenhouse topsoil (Fort Collins clay loam) study are presented in Table 53. Although no significant differences were observed between progenies for acetylene reduction at α = 0.05, there were significant differences at α = 0.10. The average rate of activity for the progenies varied substantially ranging from 14.4-88.6 μmols of ethylene evolved per gram of dry nodule per hour. In comparison to *Hedysarum* mean acetylene reduction rate from the greenhouse study on Piceance Basin soil, these data had an overall smaller mean (52.3 compared to 88.4). This may be a result of greenhouse temperature differences, time of year for the two studies, or watering regimes. More probable is the higher nitrogen status of the Fort Collins farm soil (the presence of soil nitrogen decreased nitrogenase activity) or the genetic differences among the plants used in the two studies. The average milligram of nodule per gram of root in this experiment was 77 compared with 34-45 in the other greenhouse seedling test of sweetvetch.

These are the second and third experiments in which acetylene reduction rates have been measured for seedlings of *Hedysarum boreale*, the first being by Redente and Reeves (1981). Their seedlings were larger and older (12-weeks instead of 8-weeks old), and only nodules were incubated rather than complete nodulated root systems. Their SNA rates were 2.4 and 3.8 μmols per gram nodule dry weight per hour (measured over a 19-hour incubation period in an atmosphere of 4% C_2H_2, 20% O_2, and 76% He; i.e., no N_2). The two rates were for sweetvetch with *Rhizobium*, and sweetvetch with *Rhizobium* and the mycorrhiza *Glomus*, respectively. The number of differences in technique, however, prevents any exact comparison of SNA results.

Hedysarum coronarium, a European species used as a check in this greenhouse experiment, produced 177 μmols of ethylene (SNA) which would give a rate about twice as active as the best *H. boreale* progeny. This was achived with 35 milligrams of nodule per gram of roots. We do not know if this species can survive the Piceance Basin climate, but future plantings will be made for test purposes.

Relationship of Legume Roots to the Soil/Retorted Shale Interface

A study was carried out on the Retorted Shale Successional plots of the Intensive Study Site in the Piceance Basin. On the 30 cm soil over retorted shale, 60 cm soil over retorted shale, and soil control plots, soil pits were dug and root systems excavated carefully to determine their position and growth in relation to the retorted shale layer. For all four species of legumes examined (alfalfa, cicer milkvetch, sainfoin, and sweetvetch) the roots became horizontal about 5 cm into the shale layer and pursued more of a zig-zag course, perhaps related to the larger fragments of the shale. No nodules were seen in the shale layer and very few in the soil above it. No change of

Table 53. Selected characteristics of sweetvetch progeny, including specific nodule activity (SNA). Progeny means are based on up to 15 progeny plants from each parent.

Progeny	SNA µmols C_2H_4 Evolved · g^{-1} Nodule · hr^{-1}	Average Nodule Weight/Plant (mg dry wt)	Average Root Weight/Plant (g dry wt)	mg Nodule/ g Root	Average Shoot Weight/Plant (g dry wt)	Shoot/Root Ratio	100-Seed Weight (g)
R-1-10	31.7	3.2	0.042	76.2	0.060	1.43	0.68
R-1-11	34.6	4.6	0.076	60.5	0.089	1.16	0.85
R-1-17	42.2	3.9	0.062	62.9	0.083	1.35	0.85
R-2-2	73.4	1.8	0.034	52.9	0.044	1.33	0.88
R-2-4	28.1	2.7	0.047	57.4	0.058	1.24	0.95
R-3-2	49.0	3.8	0.040	95.0	0.051	1.27	1.06
R-3-3	41.1	2.7	0.052	51.9	0.056	1.09	1.13
R-3-16	14.4	4.3	0.033	130.3	0.061	1.84	1.09
R-4-7	88.6	4.0	0.041	97.6	0.069	1.70	0.92
R-4-11	65.4	2.3	0.033	69.7	0.067	1.99	0.85
R-5-10	46.5	3.2	0.027	118.5	0.052	1.91	0.91
R-5-12	71.0	3.6	0.43	83.7	0.073	1.69	1.02
R-6-1	53.8	4.4	0.049	89.8	0.093	1.91	1.33
S-3-11	59.9	7.2	0.101	71.3	0.127	1.26	1.24
S-5-1	60.3	3.2	0.047	68.1	0.085	1.80	1.26
S-7-1	51.4	4.7	0.074	63.5	0.082	1.11-	1.03
ISS	78.3	2.0	0.030	66.7	0.037	1.26	0.74
$\bar{X}$	52.3	3.6	0.048	77.4	0.069	1.49	1.99
S.E.	4.7	1.3					

root direction was seen at any definite depth in soil layers above shale or in the soil control.

Since there is only a limited number of plants available for excavation near and in alleys of the Retorted Shale plots at ISS, more plots need to be set out on industry sites which have different types of spent shale and different climates (e.g., Parachute Creek and Anvil Points). Preliminary arrangements have been made with some of the industry personnel, and transplants for 1982 and 1983 use are being prepared in the greenhouse from seed of several legume species, including the more valuable progenies of sweetvetch.

Long-Term Fertility Study

Most schemes proposed for the vegetation stabilization of retorted oil shale prescribe using a replaced topsoil to cover the retorted material. The semiarid soils of the Piceance Basin are generally deficient in nitrogen. Since nitrogen can be limiting to the reestablishment of vegetation on a disturbed site, the management of fertility (especially nitrogen) is important to the initial success of stabilization.

Three purposes specific to fertility are to provide adequate nutrition for quick plant establishment, establish maintenance fertility levels for long-term support of plant cover, and promote the microbial and soil development processes helpful to soil stabilization.

Since nitrogen and phosphorus are generally most limiting to plant growth, availability as nutrients is of prime concern. While phosphorus deficiency limits plant establishment, sufficient levels can be maintained for several years from single, preplant application. Nitrogen, on the other hand, is difficult to maintain as an available nutrient in the root zone. Nitrogen can be lost by several mechanisms. Gaseous losses as NH_3 have been noted to be quite high in disturbed sites. The calcareous soil with a pH of about 8.2 as well as the fallow condition of the soil contribute to the problem. Denitrification has been disregarded as a major source of loss in previous progress reports because of insufficient water and organic matter for energy requirements of denitrifying bacteria. However, the fluctuating redox of a temporary perched water table and the supply of small amounts of energy in the soil could present the necessary conditions. Also, the precipitation occurs often in the summer in short, intense thunderstorms which could foster this condition. The perching of water, that is the increased water storage above a soil-shale interface, has been noted in the Retorted Shale Successional Study plots at the soil-shale interface at 60 cm. Other interfaces are created within the shale because of the method of placement. The properties of these interfaces has yet to be determined for Paraho shale (McWhorter, personal communication, 1982).

Nitrogen can also be immobilized in microbial biomass and within plant tissue without this nitrogen being lost from the internal nitrogen cycle. Immobilized N can slowly become available over a period of years as mineralization occurs. Soluble nitrogen in the form of nitrate can also be lost by percolation beyond the root zone. More than just a loss of available nitrogen, percolation represents a possible hazard in groundwater contamination.

One mechanism for maintaining N levels is to induce the immobilization of inorganic nitrogen for slow mineralization later, thus protecting the nitrogen from permanent loss from the system. Since the microorganisms which decompose organic material such as wood wastes with high C:N ratios require nitrogen for their metabolism, they use the readily available inorganic nitrogen from fertilization. Eventual mineralization of this microbial nitrogen should allow for the persistent and long-term availability of maintenance nitrogen. This study utilizes this concept in several of the fertility treatments.

The 1981 progress report (Sabey et al. 1981) indicated that nitrogen decreased significantly over time for all treatments except Treatment 15 which had a high amount of both sewage sludge and wood waste. This treatment had significantly higher levels than the control for total soil nitrogen after three years, suggesting that the sewage sludge and wood waste had a residual effect on nitrogen content. Also, no significant enhancement of plant growth was found at any nitrogen application level.

Since the fate of lost nitrogen has been a prime question suggested by this project, a study was done in late November 1981 to investigate possible losses by leaching. The potential for groundwater contamination by high nitrate levels as well as other contaminants are other reasons for monitoring the percolation of nitrate. Recent research with lysimeters with Paraho retorted shale have shown that the quality of percolate water can be extremely poor and the salt pollution potential high (Harbert 1978).

The sampling of the Topsoil Over Retorted Shale plots is the first to include the shale material in any analysis of any parameter. Other studies have considered the movement of soluble salts in Paraho shale, but nitrate has not been investigated (Harbert 1978). Since nitrate can move as slow as one-quarter the rate of soluble salts, these two parameters cannot be readily correlated (Corey 1976). This difference probably results from the unique nature of nitrogen transformations and adsorptions within the soil.

Since the depth samples were obtained in late November, the soil temperatures were quite low but the soil was not frozen. Light patches of snow remained on the ground from a recent snowfall. The topsoil was moist throughout with seemingly higher moisture contents just above the soil-shale interface. The shale material below was fairly dry however.

The analysis of variance indicated a significant difference by depth (p=.05) and a significant interaction of depth by treatment (p=.05). Least significant difference was then determined to be 10.06 ppm for differences among all depths and 12.12 ppm for the depth within treatments (Figs. 46-50).

Figures 46-50 show the NO_3 content of each depth increment of Treatments 3, 7, 11, 15, and 20. When the profile NO_3 distribution of the control (Treatment 20) and the other treatments were compared, it was evident the NO_3 movement into the shale layer had occurred. The greatest movement occurred in sewage sludge and wood waste plots (Treatment 15), but the greatest amount of NO_3 accumulating in the shale layer was in the plots treated with 896 kg N/ha at the beginning of the study (Treatment 7).

The two treatments with wood waste (Treatments 11 and 15) showed very similar profiles with no significant differences between them at any depth interval. Whereas the nitrate level in Treatment 15 with sewage sludge and wood waste differed significantly at the shale-compacted shale interface, Treatment 11 with organic N and wood waste showed no significant difference at this interface. Treatment 15 is the only fertility treatment to show such a difference.

The highest nitrate level in the compacted shale was 15.73 ppm in the initial inorganic nitrogen application (Treatment 7) where 896 kg N/ha added in a single application in 1977 (Fig. 49).

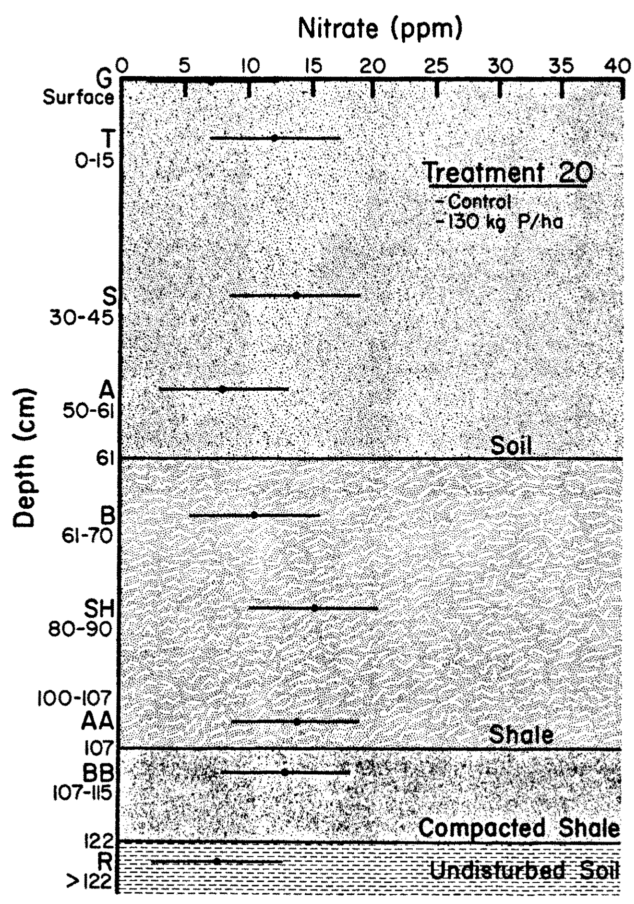


Fig. 46. Distribution of soil nitrate with depth.

added in a single application in 1977 (Fig. 49). However, there were no significant differences in nitrate content of the compacted shale among all treatments. Although Treatment 15 was the only fertility treatment with a significant difference across the shale-compacted shale interface (Fig. 50), all fertility treatments caused significant differences between the compacted shale (BB) and at least one shale interval within the uncompacted material (Figs. 47-50).

None of the nitrate levels in the undisturbed subsoil were significantly different (p=.05) from each other. The range of values was from 4.42 (Fig. 49) to 8.72 ppm (Fig. 50). This range is very similar to the indigenous nitrate levels, so very little, if any, percolation was indicated.

There are several conclusions that can be reached based on the data from this study. Nitrate did move from the layer of incorporation into the shale layer. This occurred in all the treatments sampled whether the NO_3 originated from inorganic fertilizer or from the sewage sludge used in Treatment 15 (Fig. 50).

The use of wood waste did not significantly reduce the percolation of nitrate (Figs. 49 and 50). However, the level of nitrate at the AA interval of Treatment 15 where both sewage sludge and wood waste were used was the highest of any treatment. This was significantly higher than other treatments using inorganic nitrogen alone.

The compacted shale layer may have prevented part of the nitrate from penetrating deeper. Although the profile figures showed that this would be a valid effect, the low level of NO_3 may have indicated that water may not have percolated this deep. Wymore (1974) suggested that there was little opportunity for deep percolation in the Piceance Basin because of the low precipitation and high evapotranspiration. Percolation of water may also have been slowed by textural changes within the profile (Miller 1974). The question of the effectiveness of compacted shale to prevent percolation requires further monitoring and more extensive sampling before it can be resolved.

The interface created by the soil over spent shale could restrict the movement of nitrate as well as promote the loss of nitrogen by denitrification. According to Harbert's study on Paraho shale (Harbert 1978) the textural change at the interface resulted in the soil being saturated while the shale remained dry immediately beneath that soil. Since the anaerobic conditions, that may have been created, promote denitrification, a possible loss of nitrogen may have occurred. This possibility needs further research.

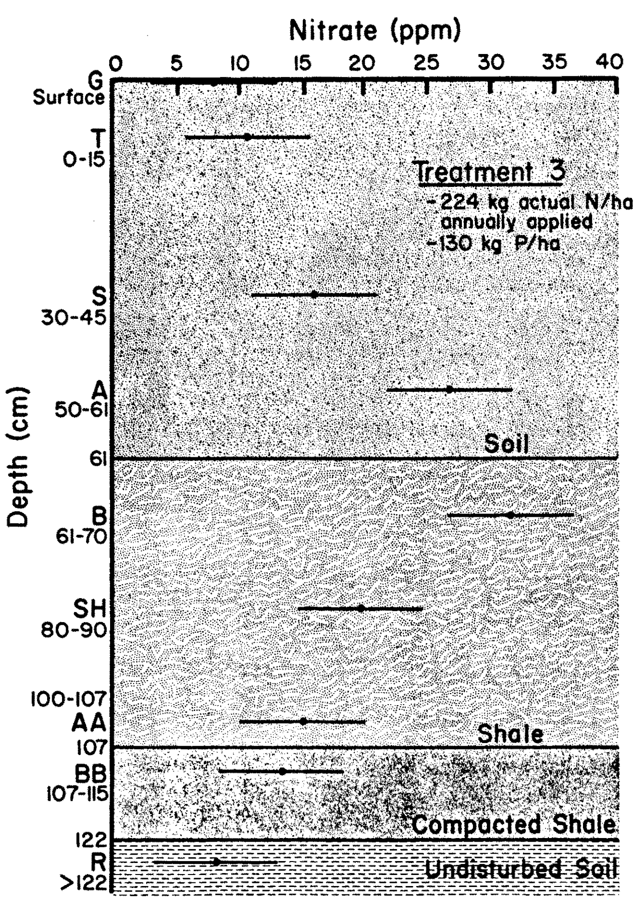


Fig. 47. Distribution of soil nitrate with depth.

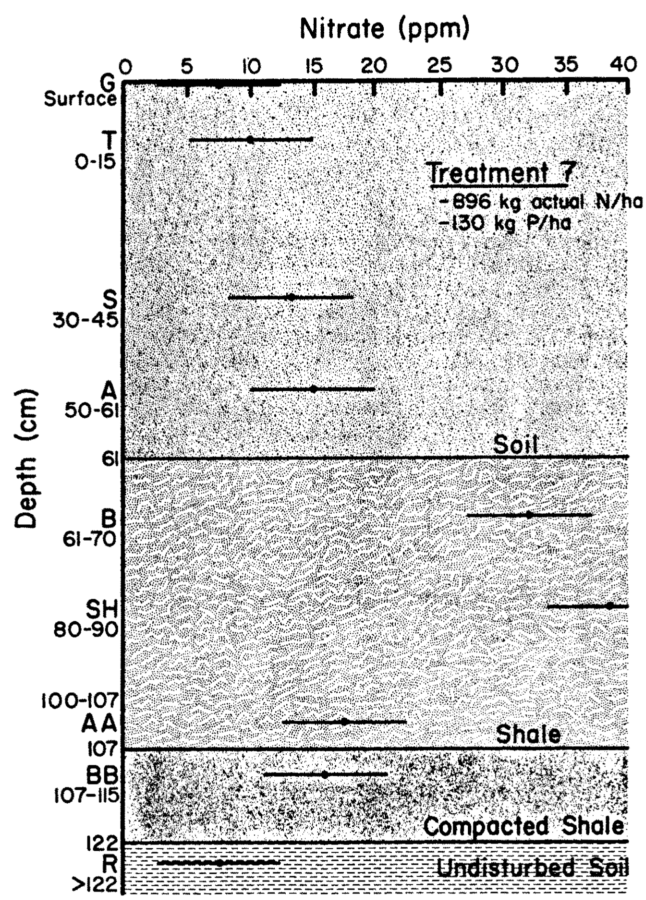


Fig. 48. Distribution of soil nitrate with depth.

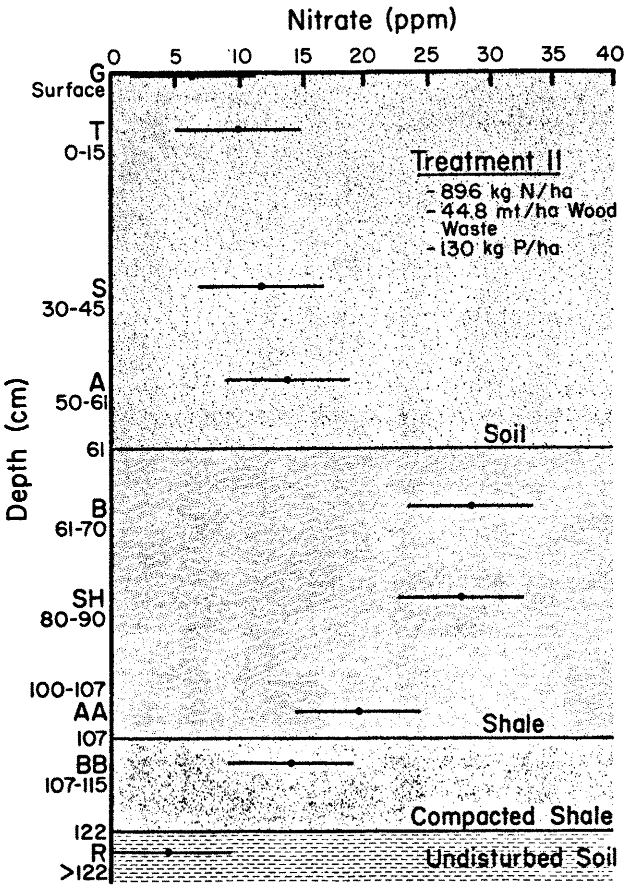


Fig. 49. Distribution of soil nitrate with depth.

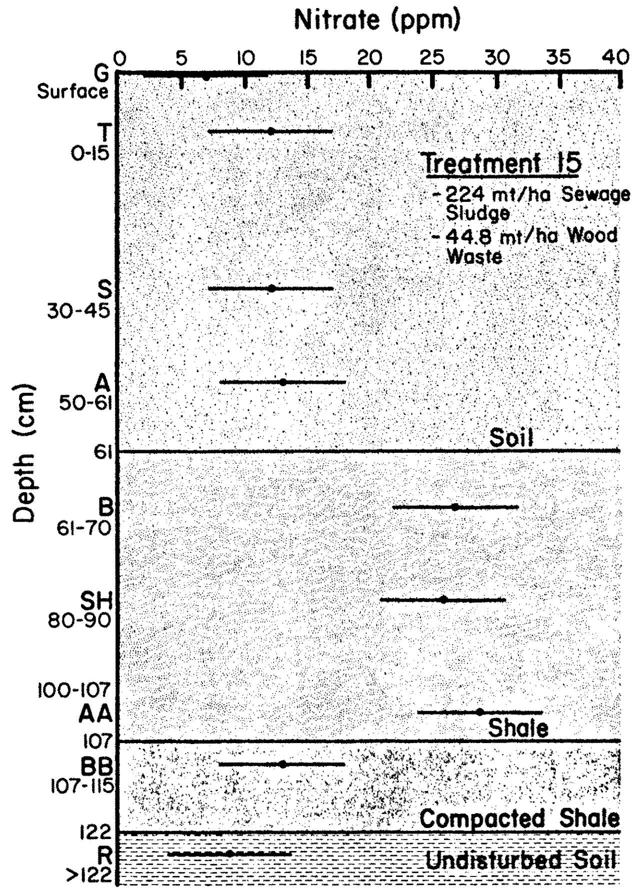


Fig. 50. Distribution of soil nitrate with depth.

This study provided evidence that downward nitrate movement has occurred in the Topsoil Over Shale plots regardless of the fertility treatment used. The question of the fate of lost nitrogen presented in previous progress reports was not resolved by this study since the nitrate levels encountered in the soil profile would not account for total losses. However, it seems clear that significant nitrate movement has resulted in substantial amounts of unavailable nitrogen. The compacted shale may be restricting the percolation of nitrate, but it is still too early to determine whether this result is from the compaction or the lack of sufficient water percolation. It is planned to sample these plots again in a similar manner to determine whether nitrate movement will continue.

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