COO-4018-3

REHABILITATION POTENTIAL AND PRACTICES OF COLORADO OIL SHALE LANDS

Progress Report for Period
June 1, 1978 - May 31, 1979

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DISCLAIMER

ABSTRACT

The following document is a third-year progress report to the original contract [Contract No. EY-76-S-02-4018] for the period June 1, 1978 to May 31, 1979. The overall objective of the project is to study the effects of seeding techniques, species mixtures, fertilizer, ecotypes, improved plant materials, mycorrhizal fungi, and soil microorganisms on the initial and final stages of reclamation obtained through seeding and subsequent succession on disturbed oil shale lands. Plant growth medias that are being used in field-established test plots include retorted shale, soil over retorted shale, subsoil materials, and surface disturbed topsoils. Because of the long-term nature of successional and ecologically oriented studies the project is just beginning to generate significant publications.

Several of the studies associated with the project have some phases being conducted principally in the laboratories and greenhouses at Colorado State University. The majority of the research, however, is being conducted on a 20 hectare Intensive Study Site located near the focal points of oil shale activity in the Piceance Basin. The site is at an elevation of 2,042 m, receives approximately 30 to 55 cm of precipitation annually, and encompasses the plant communities most typical of the Piceance Basin.

Most of the information contained in this report originated from the monitoring and sampling of research plots established in either the fall of 1976 or 1977. Therefore, data that have been obtained from the

Intensive Study Site represent only first- or second-year results. However, many trends have been identified in the successional process and the soil microorganisms and mycorrhizal studies continue to contribute significant information to the overall results. The phytosociological study has progressed to a point where field sampling is complete and the application and publication of this material will be forth coming in 1979. The plant selection and ecotype studies have made substantial progress, but because of the nature of the research publishable information is not yet available.

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INTRODUCTION

The efforts of all contributing investigators have been integrated and directed toward the goal of providing guidelines for the rehabilitation potential and practices of Colorado oil shale lands. All studies are contributing to this goal, but some projects, because of their long-term nature or sophisticated sampling and laboratory techniques, require more time to generate useful data.

All subprojects emphasize the bioedaphic factors that will be important in the rehabilitation of mined oil shale lands. The various accomplishments and significant results that have been obtained during the third year of research activities are described in this report. The introduction presents a brief summerization of the progress to date for each subproject. Following this introductory summary is detailed information on each of the studies outlined. A diagram of the Intensive Study Site is provided in Figure 1 to aid in visualizing the size and location of the field test plots discussed in this report.

Effect of Plant Species, Soil Material, and Cultural Practices Upon Plant Establishment and Succession

Major emphasis during the 1978 field season was on the monitoring and sampling of the Revegetation Technique Plot, Surface Disturbed Successional Plot, Retorted Shale Successional Plot, and the Annual Disturbance Plots (see Figure 1). A comprehensive sampling program was initiated using randomly located, permanently placed quadrats in each of

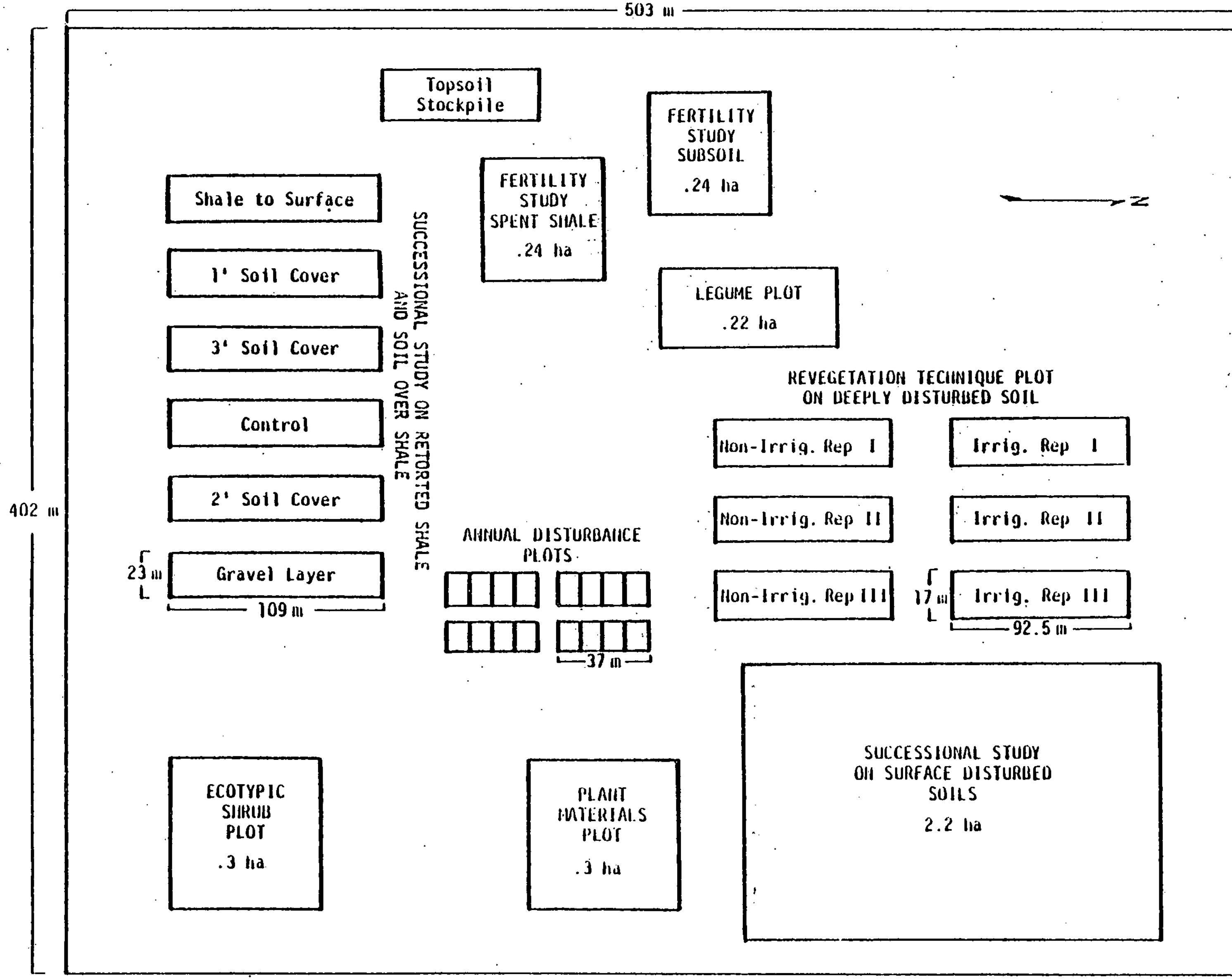

Figure 1. Diagram of the Piceance Basin Intensive Study Site presenting size and relative location of field test plots within the site.

the test plots. Primary vegetational parameters measured were density, cover, and aboveground biomass by individual plant species. Plant vigor was monitored through the measurement of maximum plant height and seed stalk production.

The Retorted Shale Successional Study (established in 1977) was sampled twice during the 1978 growing season. Initial sampling in June provided early establishment data, while sampling in August produced data on first-year survival. The remaining test plots were sampled at the period of peak production to determine second-year survival and successional trends.

In addition to the vegetational sampling, soil and retorted shale samples were analyzed for toxicity and nitrogen status. Subsurface and surface soil moisture readings were taken throughout the growing season to aid in the interpretation of the successional process.

The vegetation response on the Revegetation Technique Plot indicates substantial interactions among irrigation, fertilization, seed mixture, and seeding technique. Technique 2 (low seeding rate for grasses and forbs, high seeding rate for shrubs) should be used if both irrigation and fertilization are proposed. However, if only fertilization is used then seeding Technique 1 should be used. It was also found that shrub dominated communities can be obtained with the use of seeding Technique 2. The microbiological results on these plots indicate that with irrigation native species showed the highest levels of soil organic matter while without irrigation the introduced species produced the highest soil organic matter. From this is appears that native species may invest more energy below ground under irrigated conditions while introduced species respond with aboveground production.

The Successional Study on Surface Disturbed Soils showed that mixtures of native species are subject to successful invasion by plants characteristic of both advanced and pioneer seral stage. Introduced and combination native and introduced species tended to retard growth of invaders as measured by both biomass and cover. The microbiological studies on these plots indicated that mixtures containing introduced grasses and mixtures containing native grasses, forbs, and shrubs produced the greatest soil organic matter. This fact is directly correlated with the aboveground response for these mixtures which were tested under nonirrigated conditions.

After one growing season for the Retorted Shale Successional Plot several conclusions can be made which must be considered preliminary at this time. Without intensive management (i.e., water, fertilizer, mulch, and other soil amendments) Paraho retorted shale will not support an adequate stand of vegetation. It has also been found that the deeper the soil profiles over shale the greater the plant production because of greater soil water storage capabilities. A combination native and introduced species mixture displayed the highest productivity and allowed the least amount of invasion. The introduced mixture had the highest density of all mixtures but also allowed the greatest invasion to occur.

Over all the test plots species reaction to developmental successional patterns will become more important in the next few years as the establishment of closed communities becomes more pronounced among treatments. The spatial mix of plants is expected to change rather markedly from year to year as competition for the environmental resources becomes keener among individual species. Occupancy and dynamics in successional expressions that ultimately lead to dominance will furnish rational conclusions for successful revegetation of distrubances brought about by oil shale development.

Long-Term Fertility Maintenance on Land Drastically Disturbed by Oil Shale Development

Activities during the 1978 field season included the establishment of a Legume Study for nitrogen fixing and first-year sampling and monitoring of the Long-Term Fertility Plots on subsoil material and topsoil over retorted shale. The long-term fertility aspect of the study is examining the addition of nitrogen to the soil system from either organic or inorganic sources applied once at the initiation of the study and also annually. Vegetation sampling during 1978 was accomplished using permanently placed quadrats. Plant growth was monitored twice during the first growing season to determine initial germination and survival. Plant measurements included density, cover, biomass, and maximum height to obtain an index for vigor.

Analysis on soil samples obtained before the first growing season indicate that all the nitrogen applied as either inorganic or organic N is not being recovered. It is hoped that a more complete picture will be obtained by changes in sampling and possibly analytical procedures. Some trends have appeared in the first year's data that indicate plant growth is improved over the control by the addition of sewage sludge, low rates of inorganic N fertilizer, and high rates of phosphorus fertilizer. Effects on plant succession by fertility treatments will be examined in subsequent growing seasons.

The first-year data show some interesting trends with regard to fertility responses on subsoil versus topsoil, indicating that both soils do not respond alike to fertility treatments. Due to the nature of this study in comparing initial applications of N fertilizer with annually applied treatments publishable information is not expected until data from subsequent growing seasons is obtained.

The Legume Plot was constructed in the fall of 1978 to determine the importance of native and introduced legumes in providing nitrogen over time to deficient soil materials and their role in future reclamation work (Figure 1). Soil nitrogen levels will be monitored to study the effect of legumes in pure stands and grass-legume mixtures both with and without nitrogen fertilization. Legume species will also be examined for nitrogen fixation by acetylene reduction analysis. The experimental design is a randomized block with 14 species treatments, 3 levels of nitrogen fertilizer, and 2 soils (topsoil and subsoil) with two replications. Following construction, the soil was sampled to determine initial levels of total nitrogen, plant available P, pH, and salts.

Suitability of Native Shrubs and Forbs for Shrubland Restoration Design: Their Possible Genetic Variability and Community-Habitat Relationships

Populations of six shrub, one forb, and two grass species were transplanted into a common garden at the Intensive Study Site in the Piceance Basin. Each species is represented by different populations within the Piceance Basin and from other areas of Colorado and southern Wyoming. The purpose here is to describe and interpret the naturally occurring, ecological-genetic variation of important species in relation to the environments of different sites where they are found. This will contribute to final recommendations concerning which population characteristics need to be emphasized when selecting species mixtures for reclamation.

Following establishment in the common garden each plant was measured or rated for various characteristics, such as, leaf length and width, plant height, number of stems and leaves, phenological condition, and vigor. The environment of each population collected has also been characterized for elevation, topographic position, slope, precipitation, temperature, incoming radiation, etc. Preliminary results indicate that ecotypic differences exist within species of *Amelanchier*, *Purshia*, *Symphoricarpos*, and *Cercocarpus*.

Sampling for the phytosociological segment of this study has been completed. Eighty-four stands of vegetation were sampled during 1978 using a sampling regime giving frequencies of herbaceous species in 1x1.5 m quadrats, cover for shrub and tree species in three height classes on a line intercept, and density and basal area of tree species in 2x10 m quadrats. This information covers the range of vegetation variation of the predominant landscape units of the Colorado oil shale region.

Vegetation typical of the Intensive Study Site was sampled in deatil to provide analogue stands for research plots on the site. The analogue stands, plus their placement in the phytosociological context of the region, will give a basis for understanding the major directions of variation away from the Intensive Site--directions in which the reclamation results obtained at the Site will have to be projected.

Selection of Native Grasses and Legumes for Improved Rehabilitation

Research on this phase of the project deals primarily with the following areas: (1) collection and assembly of germplasm sources, (2) evaluation of spaced nursery plantings under controlled stress, (3) evaluation of seed progenies at the Intensive Study Site, and (4) development of improved strains of grasses and forbs for reclamation.

The evaluation of seed progenies in the Piceance Basin is one of the most important steps in determining which commercial varieties, strains, accessions, ecotypes, or single-plant progenies can succeed in the rehabilitation process.

Several strain tests are underway at the Intensive Study Site. Western wheatgrass (*Agropyron smithii*), Indian ricegrass (*Oryzopsis hymenoides*), and several leguminous species have been evaluated during the 1978 growing season. Results indicate that varieties Arriba, Mandan 456, Rosana, and C-30 are the best performers for western wheatgrass. Indian ricegrass varieties of Sharp's, Warner, M-700, and SCS's strain (NM-168) appear to provide the highest vigor, growth, and survival. Major and minor strains of lupines (*Lupinus* spp.), Utah sweetvetch (*Hedysarum boreale*), cicer milkvetch (*Astragalus cicer*), and other legumes will be tested in 1979 for their effectiveness to fix nitrogen in native soil at the Intensive Study Site.

Role of Soil Microorganisms as Indicators and Possible Controlling Factors in Plant Succession Processes on Retorted Shale and Disturbed Soils

The responses and changes in soil microbial types and activities during the reestablishment of plant communities on disturbed sites and strategies for rehabilitation by which microbe-plant interactions can be utilized are being developed in this subproject. In addition, the effects of soil storage and retorted shale on essential soil microbiological processes are being evaluated.

The Revegetation Technique Plot was sampled at the Intensive Study Site in May and July, 1979, where it was found that irrigation of the native seed mixture produced increased levels of soil organic matter while nonirrigated plots of introduced seed mixtures produced higher organic matter content in the soil. A trend towards decreased N_2 fixation potential with nitrogen fertilization additions was noted, and higher nitrogen fixation rates were found on irrigated plots as opposed to nonirrigated treatments.

All subplots were sampled on the Surface Disturbed Successional Plot during the summer of 1978. The most significant findings were that different seed mixtures and a pronounced effect on the accumulation of organic matter in soils with the introduced grass mixture and the native grass-forb-shrub mixture giving the highest levels, both with and without mulching. This response was independent of fertilization treatment.

Topsoil stored in a stockpile at the Intensive Study Site was sampled after four months of storage, where plants had not yet been established. The soil samples at the surface of the storage pile were found to have significantly lower water contents than from deeper locations, which would be expected. The surface soil also had significantly lower organic matter levels, decreased nitrogen fixation potential, lower bacterial and actinomycete populations, and higher pH values compared to topsoil stored at deeper depths.

Other experiments were also conducted on the effects of retorted oil shale on nodulation efficiency and nitrogen fixation potential by legumes and by culturing *Rhizobia* without plants present, where nitrogen fixation could take place. The plants have indicated that aboveground biomass was decreased with increasing concentrations of retorted shale. However, beyond additions of 10 to 20 percent shale the belowground portion of these plants did not show further decreases in biomass. In comparison with controls nodule production was lower with a 10 percent shale addition level, but with additional retorted shale an increase in nodule formation was observed which approached and exceeded the control soil values. *Rhizobia* cultured without plants under nitrogen fixing conditions showed stimulation with lower levels of retorted shale extracts and inhibition of nitrogen fixation with higher levels. Nitrogen fixation by *Azotobacter* was found to be sensitive to shale presence, and no stimulation of nitrogen fixation has been observed with the soil-shale mixtures examined to date.

Importance of Mycorrhizal Fungi in Salvaging and Stabilizing Disturbed Soils and Retorted Oil Shale with Native and Introduced Vegetation

This subproject is designed to determine the changes in mycorrhizal fungi following disturbance and to relate these changes in rehabilitation practices. At this point in time two major contributions have been made to the study of endomycorrhizae. The first contribution resulted from a comparison of disturbed and undisturbed sagebrush ecosystems. It was found that a strong correlation exists between the presence of viable propagules of endomycorrhizal fungi and the species composition of the plant community. This concept is extended to primary succession in harsh habitats wherein the absence of mycorrhizal fungi dictates the types of plants that can invade and survive.

The second contribution resulting from this research was the establishment of a bioassay which would better assess the actual potential of the soil to support mycorrhizal formation. The bioassay measures the active inoculum in the soil without reference to number of spores.

Other findings that have been generated by this subproject are as follows: (1) with increasing soil disturbance the mycorrhizal infection potential of the soil decreases, (2) certain traditionally nonmycorrhizal families have been found to contain a few species which appear to be mycorrhizal related under native conditions, (3) mycorrhizal fungi may be host specific, (4) the most successful revegetation species become mycorrhizal only late in their establishment, and (5) there appears to be no significant effect of seed mixture, fertilizer, mulch, or irrigation on mycorrhizal infection potential following disturbance.

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

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OBJECTIVES

This study was designed to obtain information related to seeding disturbed areas for the express purpose of reestablishing a diverse, functional ecosystem in as short a time a possible. The objectives of the research include: (1) determining proper seeding practices of potentially usable mixtures of plant species and the relationships to cultural practices and fertilizer, (2) determining rate and direction of plant succession on overburden material and retorted shale overlain by soil as influenced by mixtures of species used in revegetation, cultural practices, and soil conditions, and (3) determining germination and establishment requirements of important native plants in growth chamber experiments.

PROGRESS TO DATE

Revegetation Techniques on Intensively Disturbed Soils

The Revegetation Technique Plot on Intensively Disturbed Soils was constructed in late summer 1976 (see Progress Report, March 1978). The configuration of the plot with the various combinations of treatments is shown in Figure 2. The treatments were as follows:

a. Irrigation

I --irrigate
NI--nonirrigate

b. Fertilization

F --fertilize (112 kg N/ha, 90 kg P/ha)
NF--nonfertilize

c. Seeding Technique

Technique 1--Drill mixtures of grasses, forbs, and shrubs

Technique 2--Drill mixtures of grasses, forbs, and shrubs with decreased seeding rate of grasses and forbs and increased rate of shrubs

Technique 3--Drill grasses and forbs, but transplant shrubs

Technique 4--Broadcast grasses and forbs, but transplant shrubs

d. Seed Mixture

Mixture 1--Combination (native and introduced) species

1.	Nordan crested wheatgrass	*Agropyron cristatum*
2.	Siberian wheatgrass	*A. sibiricum*
3.	Critana thickspike wheatgrass	*A. dasystachyum*
4.	Sodar streambank wheatgrass	*A. riparium*
5.	Slender wheatgrass	*A. trachycaulum*
6.	Regar meadow brome	*Bromus erectus*
7.	Indian ricegrass	*Oryzopsis hymenoides*
8.	Green needlegrass	*Stipa viridula*
9.	Durar hard fescue	*Festuca ovina duriuscula*
10.	Madrid yellow sweetclover	*Melilotus officinalis*
11.	Utah sweetvetch	*Hedysarum boreale utahensis*
12.	Globemallow	*Sphaeralcea munroana*
13.	Lewis flax	*Linum lewisii*
14.	Arrowleaf balsamroot	*Balsamorhiza sagittata*

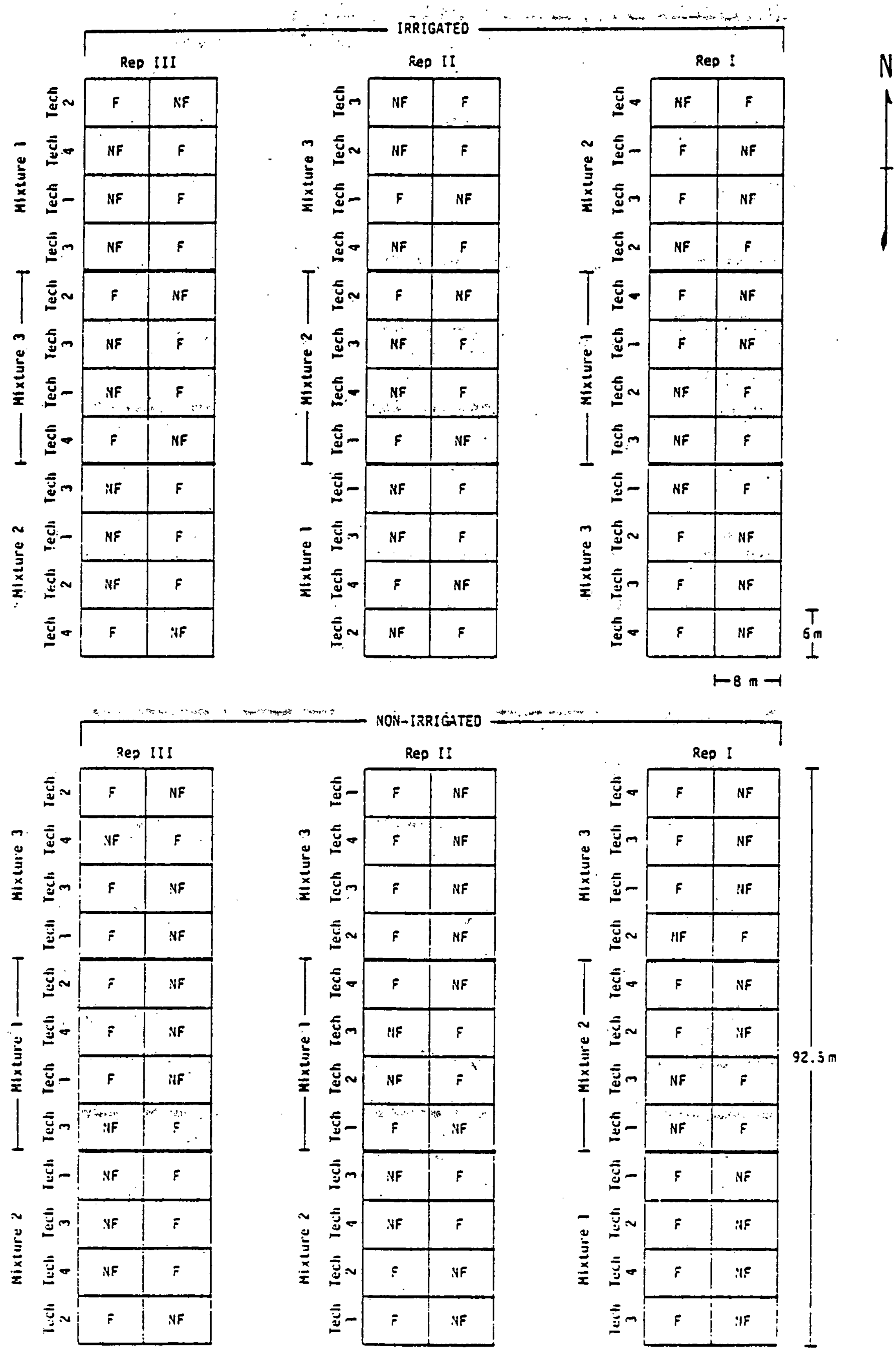

Figure 2. Experimental design for the Revegetation Technique Plot on Intensively Disturbed Soils.

15. Fourwing saltbush — *Atriplex canescens*
16. Stansbury cliffrose — *Cowania mexicana stansburiana*
17. Winterfat — *Ceratoides lanata*
18. Green ephedra — *Ephedra viridis*

Mixture 2--Native species

1. Rosana western wheatgrass — *Agropyron smithii*
2. Sodar streambank wheatgrass — *A. riparium*
3. Bearded bluebunch wheatgrass — *A. spicatum*
4. Indian ricegrass — *Oryzopsis hymenoides*
5. Green needlegrass — *Stipa viridula*
6. Durar hard fescue — *Festuca ovina duriuscula*
7. Shermans big bluegrass — *Poa ampla*
8. Alkali sacaton — *Sporobolus airoides*
9. Globemallow — *Sphaeralcea munroana*
10. Utah sweetvetch — *Hedysarum boreale utahensis*
11. Palmer penstemon — *Penstemon palmeri*
12. Stansbury cliffrose — *Cowania mexicana stansburiana*
13. Green ephedra — *Ephedra viridis*
14. Fourwing saltbush — *Atriplex canescens*
15. Winterfat — *Ceratoides lanata*
16. Antelope bitterbrush — *Purshia tridentata*

Mixture 3--Introduced species

1. Nordan crested wheatgrass — *Agropyron cristatum*
2. Siberian wheatgrass — *A. sibericum*
3. Jose tall wheatgrass — *A. elongatum*
4. Luna pubescent wheatgrass — *A. trichophorum*
5. Oahe intermediate wheatgrass — *A. intermedium*
6. Manchar smooth brome — *Bromus inermis*
7. Regar meadow brome — *B. erectus*
8. Vinal Russian wildrye — *Elymus junceus*
9. Ladak alfalfa — *Medicago sativa*
10. Madrid yellow sweetclover — *Melilotus officinalis*
11. Lutana cicer milkvetch — *Astragalus cicer*
12. Sainfoin — *Onobrychis viciaefolia*
13. Bounding bet — *Saponaria officinalis*
14. Small burnet — *Sanguisorba minor*
15. Siberian peashrub — *Caragana arborescens*
16. Russian olive — *Elaeagnus angustifolia*

Seeding rates for the above mixtures are shown in Table 1.

During the 1978 field season six permanently located, rectangular-shaped 0.25 m^2 quadrats were established in each subplot (Figure 2) using random coordinates and a grid system. The quadrats were marked in opposite corners with steel stakes. These quadrats will continue to be

Table 1. Seeding rates for Revegetation Technique Plot on Intensively Disturbed Soils (pounds per acre of PLS).

Species	Tech 1	Tech 2	Tech 3	Tech 4
Mixture #1--Combination				
1. Nordan crested wheatgrass	1.0	0.5	1.0	2.0
2. Siberian wheatgrass	1.0	0.5	1.0	2.0
3. Critana thickspike wheatgrass	1.0	0.5	2.0	3.0
4. Sodar streambank wheatgrass	1.0	0.5	2.0	3.0
5. Slender wheatgrass	1.0	0.5	2.0	3.0
6. Regar meadow brome	1.0	0.5	1.0	2.0
7. Indian ricegrass	1.0	0.5	2.0	2.0
8. Green needlegrass	1.0	0.5	2.0	3.0
9. Durar hard fescue	0.5	0.25	0.5	1.0
10. Madrid yellow sweetclover	0.5	0.25	0.5	0.5
11. Utah sweetvetch	1.0	0.5	1.0	1.5
12. Globemallow	0.5	0.5	0.5	1.0
13. Lewis flax	0.5	0.5	0.5	1.0
14. Arrowleaf balsamroot	1.0	0.5	1.0	1.5
15. Fourwing saltbush	1.0	4.0	---	---
16. Stansbury cliffrose	1.0	3.0	---	---
17. Winterfat	1.0	2.0	---	---
18. Green ephedra	1.0	2.0	---	---
TOTAL	16.0	17.5	17.0	26.5
Mixture #2--Native species				
1. Rosana western wheatgrass	1.0	0.5	3.0	4.0
2. Sodar streambank wheatgrass	1.0	0.5	1.0	2.0
3. Bearded bluebunch wheatgrass	1.0	0.5	2.0	4.0
4. Indian ricegrass	1.0	0.5	2.0	4.0
5. Green needlegrass	1.0	0.5	2.0	4.0
6. Durar hard fescue	0.5	0.25	0.5	1.0
7. Shermans big bluegrass	1.0	0.5	1.0	1.0
8. Alkali sacaton	0.5	0.25	0.5	1.0
9. Globemallow	0.5	0.25	0.5	1.0
10. Utah sweetvetch	1.0	0.5	1.0	1.0
11. Palmer penstemon	0.5	0.25	0.5	1.0
12. Stansbury cliffrose	2.0	4.0	---	---
13. Green ephedra	1.0	3.0	---	---
14. Fourwing saltbush	1.0	3.0	---	---
15. Winterfat	1.0	2.0	---	---
16. Antelope bitterbrush	1.0	3.0	---	---
TOTAL	15.0	19.5	14.0	24.0

Table 1.--Continued

Species	Tech 1	Tech 2	Tech 3	Tech 4
Mixture #3--Introduced species				
1. Nordan crested wheatgrass	1.0	0.5	2.0	3.0
2. Siberian wheatgrass	1.0	0.5	1.0	2.0
3. Jose tall wheatgrass	1.0	0.5	2.0	3.0
4. Luna pubescent wheatgrass	1.0	0.5	1.0	2.0
5. Oahe intermediate wheatgrass	1.0	0.5	1.0	2.0
6. Manchar smooth brome	1.0	0.5	1.0	2.0
7. Regar meadow brome	1.0	0.5	2.0	4.0
8. Vinal Russian wildrye	1.0	0.5	1.0	2.0
9. Ladak alfalfa	0.5	0.25	0.5	1.0
10. Madrid yellow sweetclover	0.5	0.25	0.5	1.0
11. Lutana cicer milkvetch	0.5	0.5	0.5	1.0
12. Sainfoin	0.5	0.5	0.5	1.0
13. Bouncing bet	1.0	1.0	1.0	2.0
14. Small burnet	1.0	1.0	1.0	2.0
15. Siberian peashrub	1.0	4.0	---	---
16. Russian olive	2.0	4.0	---	---
TOTAL	15.0	15.5	15.0	28.0

used in subsequent years to measure parameters associated with population dynamics of individual species. Primary parameters were number, cover, and biomass for individual species and life forms. These measurements were made on both seeded and invading species. Invading species were identified as any plant not included in the original seeding mixtures. Biomass was obtained by correlating nonconsumption estimates from the subplots with estimates and clipped plots taken from the buffer zones. Other measured parameters were maximum plant height and seed stalk production. Seed stalk production was measured by counting the number of seed stalks per species per quadrat. Maximum plant height was determined in centimeters from average ground level to top of highest leaf or stem.

Statistical analysis of field data tested the null hypothesis that irrigation treatments, fertilizer applications, seed mixtures, seeding techniques, and mulch treatment do not affect the population parameters measured. Differences between means were tested by use of Tukey's Q-test.

Beginning the first week in June, supplemental irrigation water was applied weekly on the irrigated subplots. The amounts varied according to the amount of natural precipitation occurring. Enough supplemental water was applied so that a total of 2.5 cm (1 inch) was supplied to the subplots each week (natural and artificial applications combined). This amount approximated the highest amount of precipitation that could be expected to occur at this site based on 20 years of previous data.

Shrub seedlings will be transplanted into subplots seeded by Techniques 3 and 4 (see Progress Report, March 1978) in May 1979. Both techniques call for establishment of forbs and shrubs by direct seeding, with subsequent establishment of shrubs by transplanting.

Results and Discussion

On the Revegetation Technique Plot four planting techniques were used which had a confounding effect upon the results dealing with establishment of life forms. Technique 1 consisted of drilling each mixture with grasses and forbs at a higher rate than shrubs (Table 1). In Technique 2 shrubs were seeded at a higher rate than grasses and forbs. The purpose of the difference in seeding rates was to reduce competition from rapidly growing grasses and forbs and allow shrub species to become established. As expected, the results support this hypothesis.

Differences in Techniques 3 and 4 are in the method of seeding grasses and forbs. These life forms were drill seeded in Technique 3 and broadcast seeded in Technique 4. Shrub seedlings will be transplanted in Techniques 3 and 4 during spring of 1979. Therefore, the results will reflect this difference in planting procedures. Absence of shrubs was the reason that data collected from Techniques 3 and 4 were not included in the analysis of variance for biomass and cover of total grasses, forbs, and shrubs.

Special consideration was given to invading species during data collection in 1978. The success of invading species is an important indicator of successional patterns. As additional data are gathered in future years, changes in the composition of invading plants will aid in the identification of species mixtures most able to approximate undisturbed native ecosystems. The native species mixture seeded on the Revegetation Technique Plot allowed the greatest amount of invasion to occur. Subplots seeded with the native mixture are therefore open communities at the present time. This indicates that the seeded native species are not fully utilizing the resources available on the site. The introduced and combination native and introduced mixtures have allowed considerably less invasion than the native mixture. From this observation it appears that subplots seeded with these mixtures are approaching closed communities more rapidly than the native mixture. The importance of open versus closed communities in reestablishing a functional and diverse ecosystem is not fully understood at the present time.

Effect of Seeding Technique on Aboveground Biomass

Total Biomass of Seeded Species. No significant difference was found between the main effect of Technique 1 and 2 for the total biomass of seeded species. However, subplots seeded with Technique 2 supported a slightly higher biomass than those subplots seeded with Technique 1. No significant difference was found between Techniques 3 and 4 (comparing drilling and broadcasting) when comparing the total biomass of seeded grasses and forbs. However, subplots drill seeded supported higher biomass than subplots seeded by broadcasting.

For the total biomass of grasses and forbs of seeded species, there was a significant interaction among irrigation, seeding technique, and fertilizer treatments (Appendix A, Table 1). As seen in Figure 3 there is an overall increase in biomass when either supplemental water or fertilizer is applied. However, the increase due to fertilization under nonirrigated conditions is only minimal. Subplots seeded with Techniques 2 and 3 displayed dramatic increases in grass and forb biomass of seeded species when irrigated and fertilized with Technique 3 being the highest.

FERTILIZATION x SEEDING TECHNIQUE x IRRIGATION

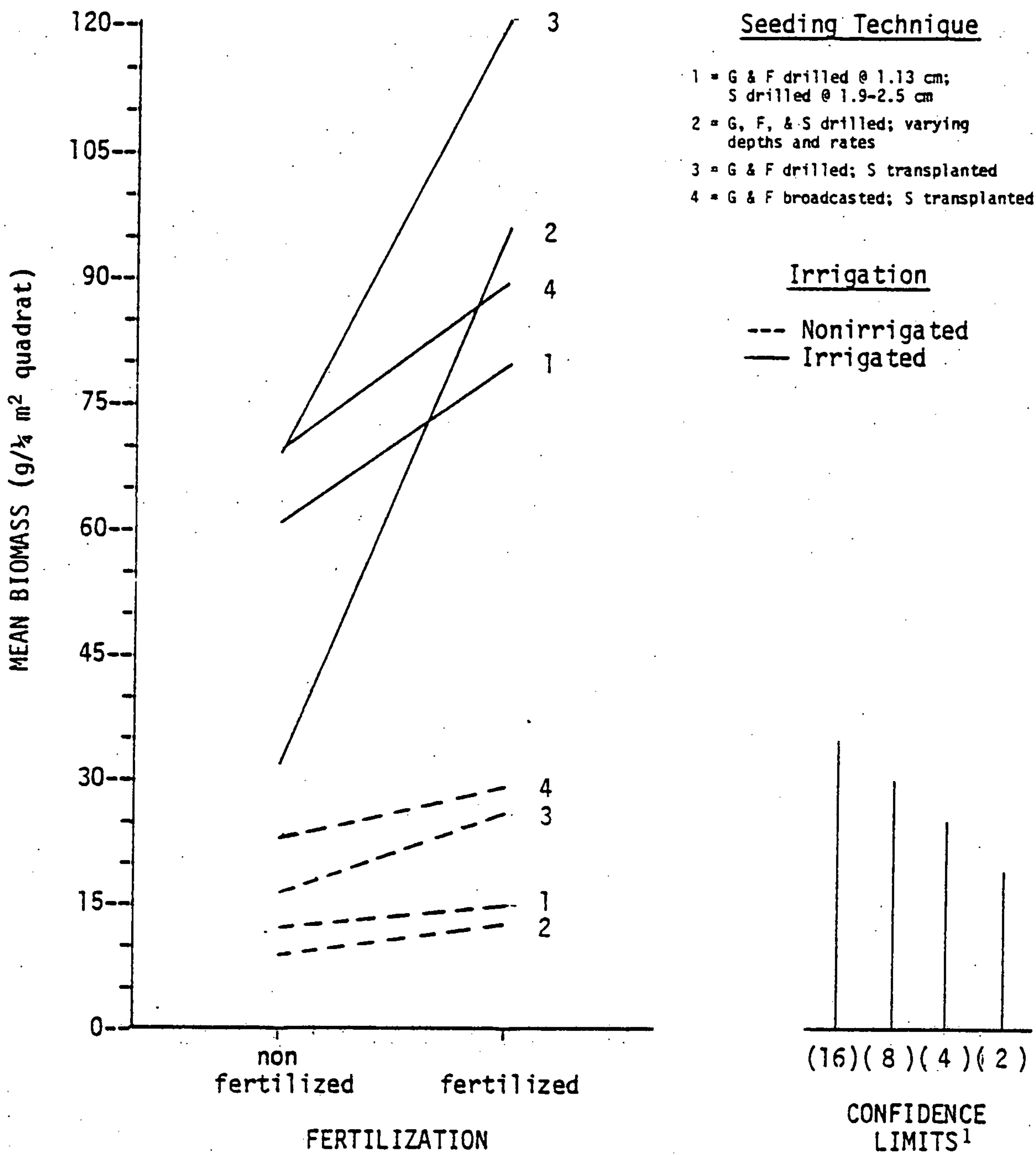

Figure 3. Mean biomass of grasses and forbs of seeded species in response to fertilization, seeding technique, and irrigation for the Revegetation Technqiue Plot on Intensively Disturbed Soils.

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[1]The honest significant difference procedure or Tukey's Q-test was used to determine significant differences between means in a significant main effect or interaction. Confidence intervals are shown above the number of means to be compared. Within interactions a group of means representing all levels of one treatment and at one level of the other treatments can be compared using the confidence interval above the number of means in the group.

From the data it appears that Techniques 2 or 3 should be used if both irrigation and fertilization are planned. However, if only fertilizer is considered in the reclamation plan, Techniques 3 and 4 should be used.

Total Biomass of Invading Species. When evaluating the total biomass of only invading species no significant main effects or interactions for the various seeding techniques were found.

Total Biomass of Grasses. There was a significant main effect for seeding technique when considering the biomass of only the seeded grass species (Appendix A, Table 2). Subplots seeded with Technique 3 supported the highest grass biomass across all three seed mixtures (Figure 4). Technique 4 which was a broadcasting procedure (with the same seed mixture but twice the quantity of seed) resulted in the second highest grass biomass production. Although the difference was not significant, it does suggest that drilling might be better than broadcasting even though more seed was planted with broadcasting. As would be expected grasses were the lowest in Technique 2 because of the lower seeding rate of grasses in this technique.

Across all four seeding techniques biomass production of seeded grass species was greatest for the seed mixture composed of introduced plants. The combination native and introduced species mixture ranked second in seeded grass biomass followed by the native mixture.

Technique 3 responded better to the addition of fertilizer and supplemental water than other techniques (Figure 5). Across all four seeding techniques grass production responsed with only a slight increase when fertilizer was added without supplemental water. However, there was a marked increase in grass production on subplots receiving both

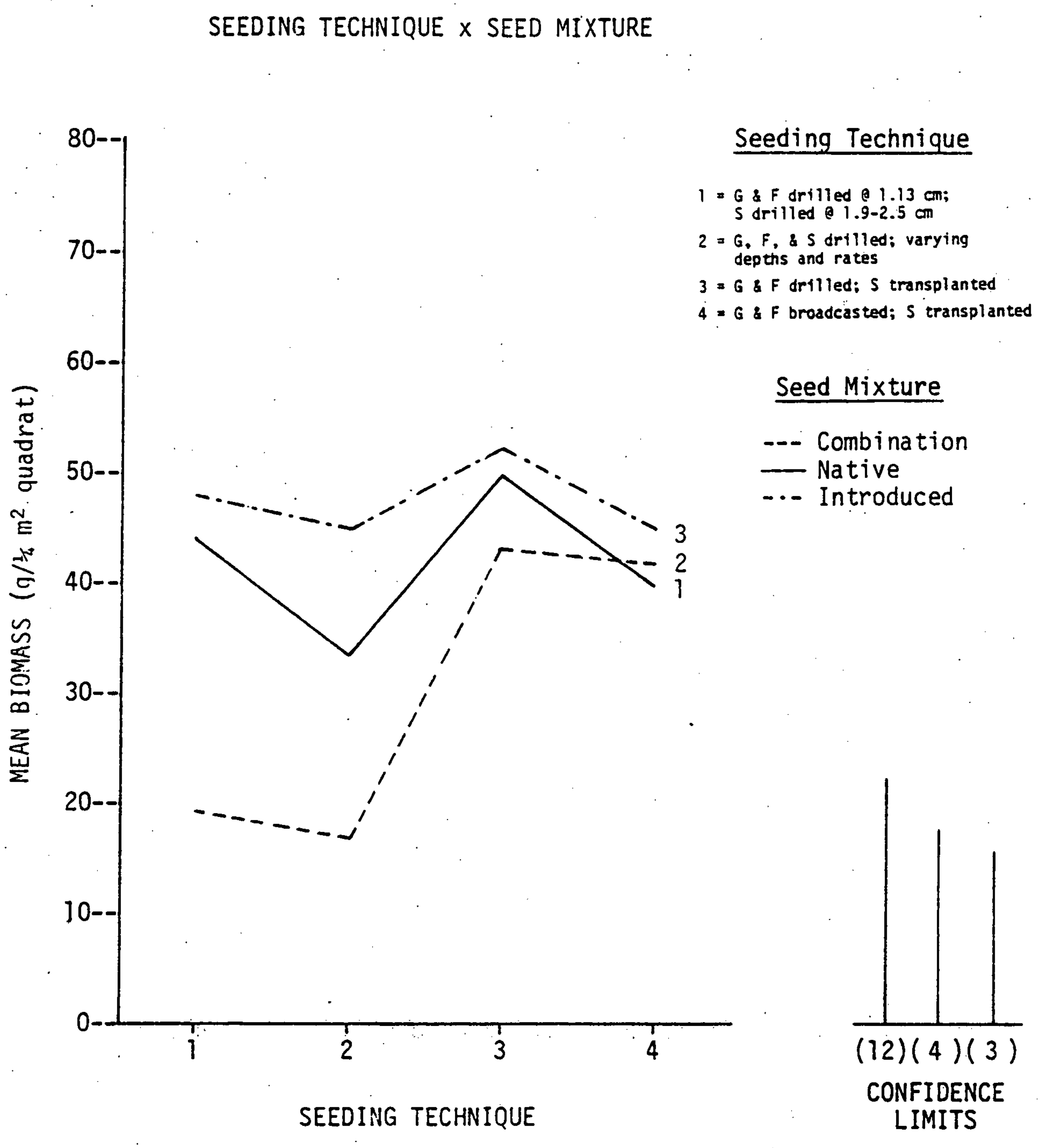


Figure 4. Mean biomass of grasses of seeded species in response to seeding technique and seed mixture for the Revegetation Technique Plot on Intensively Disturbed Soils.

FERTILIZATION x IRRIGATION x SEEDING TECHNIQUE

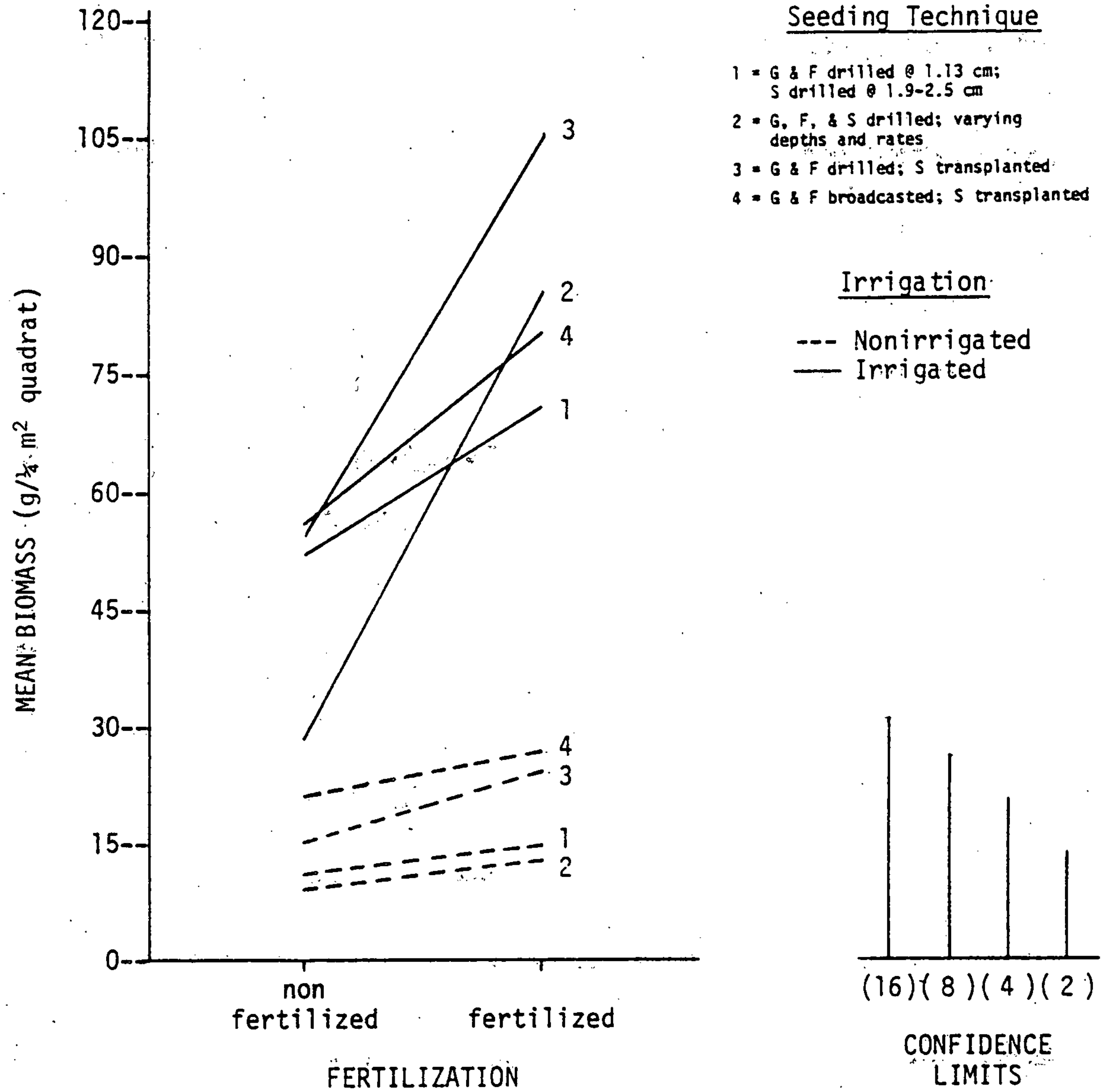


Figure 5. Mean biomass of grasses of seeded species in response to fertilization, irrigation, and seeding technique for the Revegetation Technique Plot on Intensively Disturbed Soils.

fertilizer and water. This was especially evident for biomass of grasses seeded with Techniques 2 and 3. The tremendous response to fertilization and irrigation on subplots seeded with Technique 2 may be caused by the lower seeding rate of grasses (reduced competition) which may have enabled individual grass plants to better utilize the added water and nutrients.

The data suggests that if irrigation is planned in the reclamation scheme, then fertilizer should also be included for maximum grass production. However, if irrigation is not feasible, the addition of fertilizer may not significantly increase grass production.

Total Biomass of Forbs. No significant main effects or interactions for the various seeding techniques were found when evaluating the biomass of seeded forbs. There were, however, some differences for invading forbs among the four seeding techniques (Appendix A, Table 3). Subplots seeded with Technique 2 allowed the greatest invasion of forbs while the broadcast seeding technique (Technique 4) allowed the least invasion.

Total Biomass of Shrubs. The overall shrub biomass of seeded and invading species was greatest on subplots seeded with Technique 2 as was expected because of the greater quantity of shrub seed planted. The amount of invading shrubs was quite small and did not make a substantial contribution to the total.

For some unknown reason the seeded shrub component on subplots planted with Technique 1 was significantly reduced under irrigated conditions (Figure 6). This possibly could have been the result of the rapid response of grasses to supplemental water which caused increased competition between grasses and shrubs. When subplots seeded with Technique 2 were irrigated, the lower grass seeding rate allowed shrubs

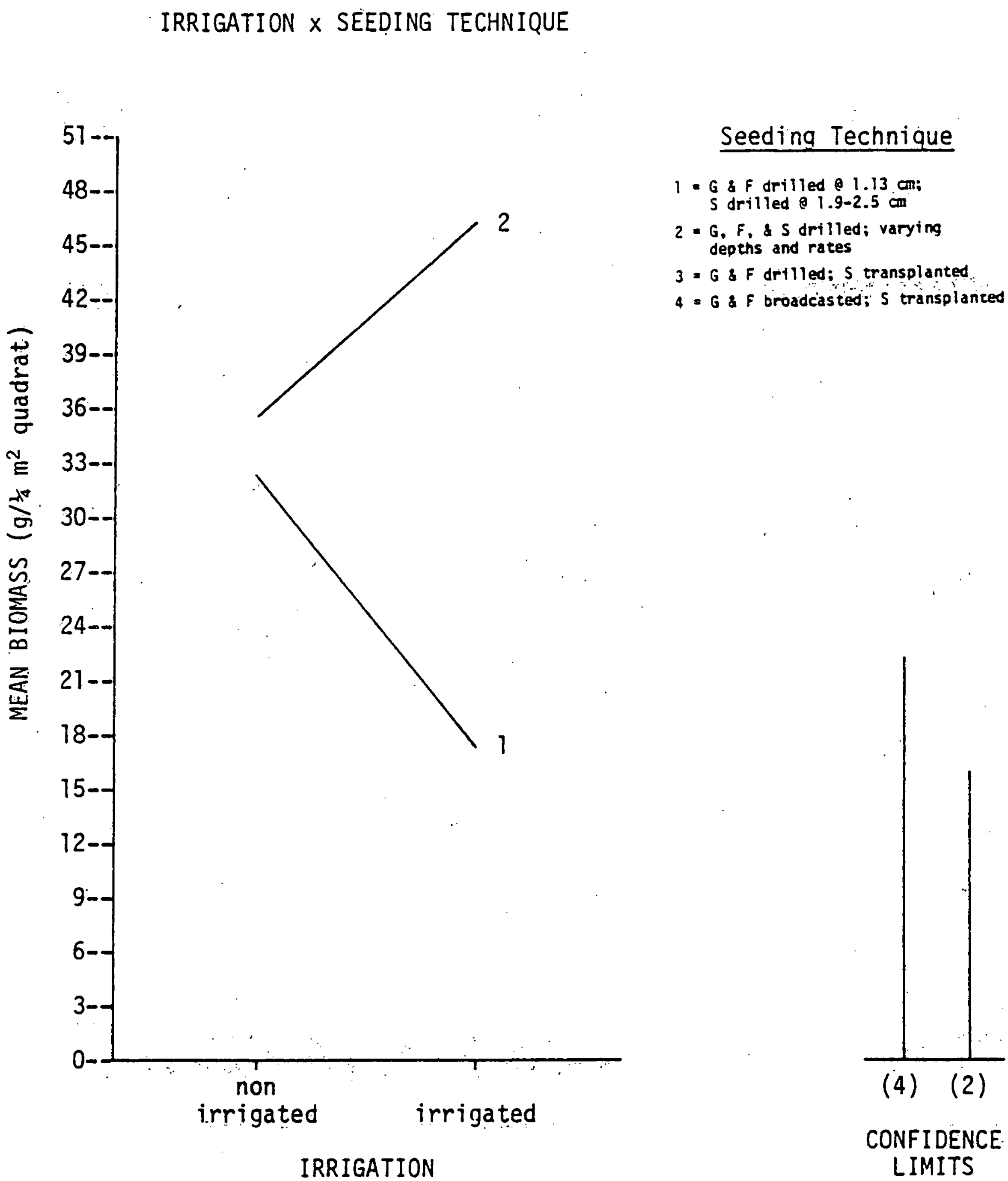


Figure 6. Mean biomass of shrubs of seeded species in response to irrigation and seeding technique for the Revegetation Technique Plot on Intensively Disturbed Soils.

to respond favorably to the added water without interference from the grass component.

Effect of Seeding Technique on Ground Cover

Total Cover of Grasses. The response of seeded grasses to planting techniques was similar for total cover when compared to total biomass. Subplots seeded with Techniques 3 and 4 supported grasses with significantly higher cover values than Techniques 1 and 2.

Across all seeding techniques fertilizer increased the percentage ground cover of seeded grasses. Cover of seeded grass species was further increased with the addition of water (Figure 7).

Effect of Seed Mixture on Aboveground Biomass

Total Biomass of Seeded Species. Although no significant differences in total biomass have been found between the three seeded mixtures, a definite trend has been noted among treatments. Subplots seeded with the combination native and introduced mixture responded with greater biomass than either the native or introduced mixture. The biomass associated with the combination mixture was composed almost entirely of seeded species. Subplots seeded with the native mixture ranked second in total biomass production, but allowed the greatest biomass of invading species. The introduced mixture, which was expected to show the greatest biomass response, exhibited the lowest total production of the three seeded mixtures. Until species information is analyzed the interpretation of these results is somewhat premature.

Total Biomass of Invading Species. There was a significant interaction for the total biomass of grasses, forbs, and shrubs of invading species among seed mixture, fertilization, and irrigation.

FERTILIZATION x SEEDING TECHNIQUE x IRRIGATION

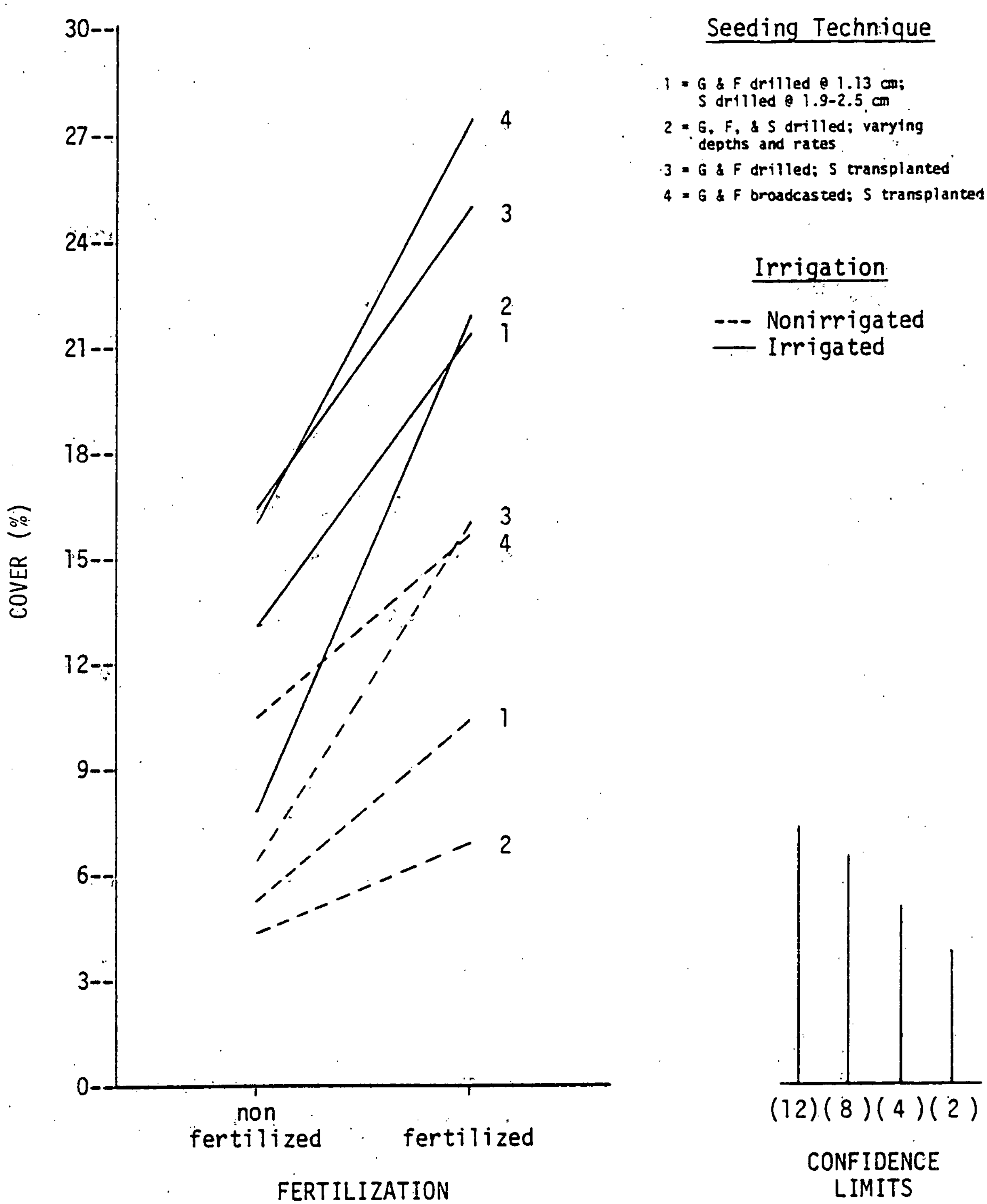


Figure 7. Percentage cover of grasses of seeded species in response to fertilization, seeding technique, and irrigation for the Revegetation Technique Plot on Intensively Disturbed Soils.

Subplots seeded with the native seed mixture allowed significantly greater invasion to irrigation and fertilization than subplots seeded with the other two mixtures. There was an intermediate response of total biomass of invading species on subplots seeded with the introduced mixture and no response on subplots seeded with the combination mixture under irrigated and fertilized conditions. Invading species responded primarily to the addition of supplemental water and showed little reaction to the addition of only fertilizer. The large amount of invasion on subplots seeded with the native mixture suggest that these subplots are open and seeded species are not fully utilizing the available resources of light, water, and nutrients.

Total Biomass of Seeded Grasses. As can be seen in Figure 8 grass biomass production of seeded species is highest on those subplots seeded with the introduced mixture. This response is contrary to the results presented earlier for total biomass of seeded species. It was noted that subplots seeded with the introduced mixture exhibited the lowest total production of any of the three mixtures. This difference between total biomass and the biomass of only the seeded grasses was caused by the failure of introduced shrubs to become established. Therefore, the lower biomass of the introduced seed mixture can be directly attributed to the failure of introduced shrubs (Russian olive and Siberian peashrub).

Total Biomass of Forbs. Subplots that received supplemental water responded with higher seeded forb biomass than those without additional water. The seeded forb component on subplots seeded with the introduced and combination mixtures responded dramatically under irrigated conditions (Figure 9). It appears that this increase in forb biomass is caused by

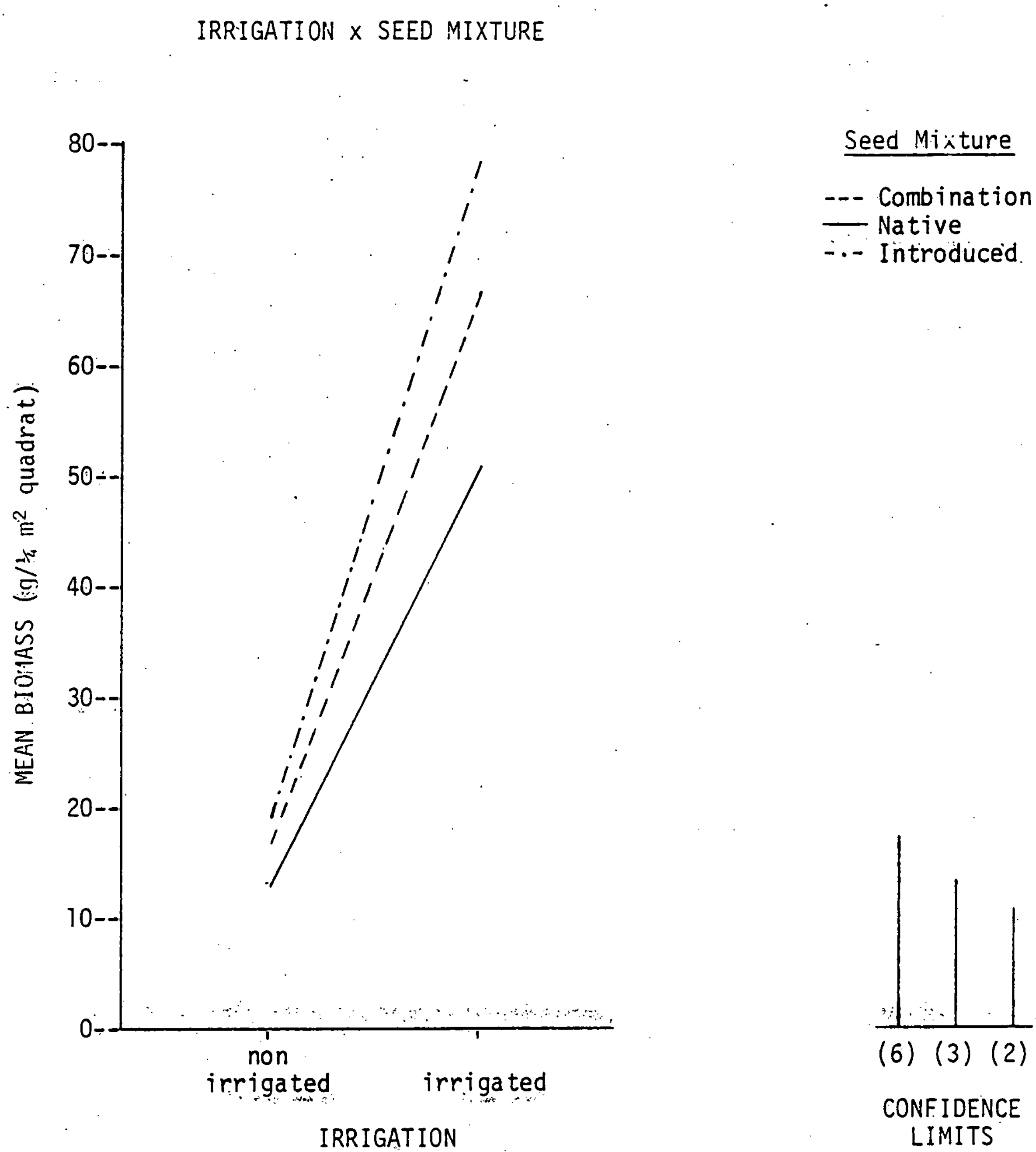


Figure 8. Mean biomass of grasses of seeded species in response to irrigation and seed mixture for the Revegetation Technique Plot on Intensively Disturbed Soils.

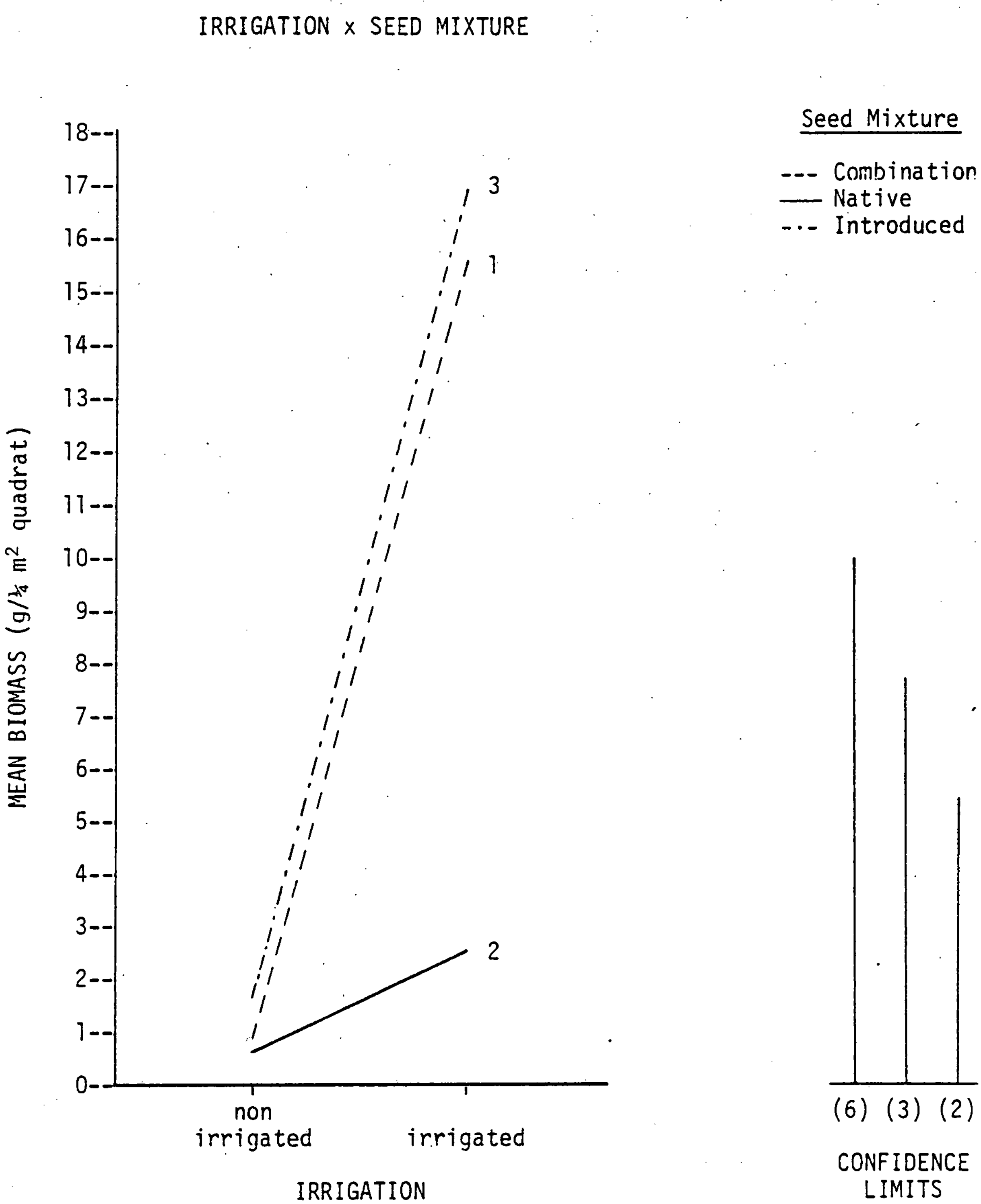


Figure 9. Mean biomass of forbs of seeded species in response to irrigation and seed mixture for the Revegetation Technique Plot on Intensively Disturbed Soils.

the favorable response of introduced forbs in these mixtures. The native forbs, however, responded only slightly with the addition of water.

Total Biomass of Shrubs. As stated previously the introduced shrubs failed to become established on subplots seeded with the introduced mixture. Therefore, there was a significantly greater production of shrubs on subplots seeded with the native and combination seed mixtures (Appendix A, Table 2).

Effect of Seed Mixture on Ground Cover

As might be expected a strong correlation was seen between the responses of biomass and cover across all treatments. This phenomenon is well documented in the literature and has held true under most treatments tested in this study. A detailed discussion of the response of cover to the various treatments tested would, therefore, only duplicate findings reported under the biomass section.

Effect of Fertilization on Aboveground Biomass

Total Biomass of Seeded and Invading Species. Plant species fertilized with nitrogen and phosphorus showed a significantly higher biomass response than those not fertilized. Fertilization and supplemental water when combined further increased the response of seeded and invading species over all treatments. Many other interactions occurred with fertilization and have been reported in previous sections.

The fact that additions of fertilizer and water significantly increased the response of seeded and invading species does not prove that they are absolutely necessary for the establishment of diverse and productive ecosystems. The data at present cannot answer the question of whether fertilizer and water are required to establish diverse plant communities on the disturbed sagebrush and juniper ecosystems of the Piceance Basin.

Conclusions

Several conclusions can be made from the data collected on the Revegetation Technique Plot. These conclusions are drawn from data collected during the second growing season and should, therefore, be considered preliminary. The dynamics of individual species as influenced by competitive pressures will be more definitive when plant communities approach closed stands and environmental resources are more fully utilized.

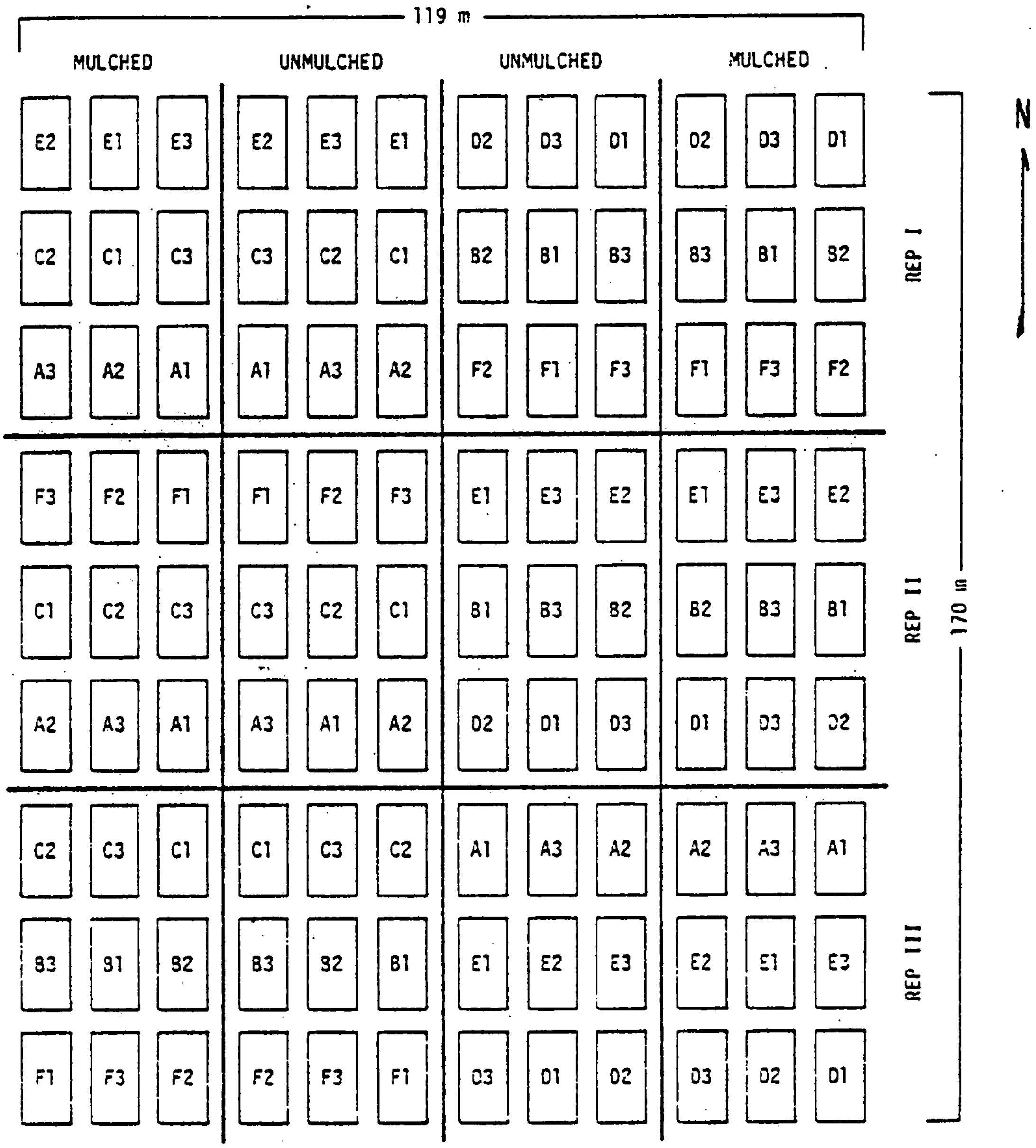
1. Technique 2 (low seeding rate for grasses and forbs, high seeding rate for shrubs) should be used if both irrigation and fertilization are proposed. However, if only fertilization is used, then Technique 1 should be implemented.
2. Shrub dominated communities can be obtained with the use of Seeding Technique 2 where the quantity of shrub seed is increased in the mixture.
3. When considering the total biomass of grasses, drill seeding proved superior to broadcast seeding.
4. Biomass production of seeded grass species was greatest for the introduced seed mixture.
5. Irrigation increased biomass and cover of seeded plants, but the response to irrigation was increased significantly with the addition of nitrogen and phosphorus.
6. Plant species fertilized with nitrogen and phosphorus showed a significantly higher biomass response than those not fertilized.

Successional Study on Surface Disturbed Soils

The Successional Study on Surface Disturbed Soils is designed to provide detailed information about the rate and direction of natural plant succession on disturbed lands in the Piceance Basin. Of special interest is the interplay between the level of fertilizer application, the species seeded in a reclamation effort, and the presence or absence of mulch on the seeded plots. Each of these factors, or several factors acting in concert, could affect the type of plant communities that reestablish following a disturbance. For example, crested wheatgrass (*Agropyron cristatum*) is known as a valuable plant for rapidly reestablishing grass cover, yet this species may retard the establishment of other components of advanced seral stages. Other species mixtures may be slower in attaining optimal biomass or cover, yet proceed toward a climax more rapidly.

In order to ascertain the optimal combination of seeded species, fertilizer, and mulch a split-split block designed experiment (Figure 10) was initiated in the fall of 1976. The area on 2.2 ha in the northeast corner of the Intensive Study Site was scraped free of vegetation, ripped to a depth of 30 cm with a D-8 caterpillar, and then rototilled. One hundred eight sub-subplots measuring 9x18 m were then delineated as shown in Figure 10. Because of the difficulty of applying the wood fiber hydromulch this treatment had to be confined to the margins of the experimental area (Figure 10).

Subplot treatments consist of six species mixtures given in Table 2. These mixtures represent the range from simple grass combinations to a relatively complex mixture of native and introduced grasses, forbs, and



MULCH

Hydro mulch
Control (unmulched)

FERTILIZER

1 = 112 kg N, 56 kg P per hectare
2 = 56 kg N, 28 kg P per hectare
3 = control (0 kg N, 0 kg P per hectare)

SPECIES SEEDED

A = native grass mixture
B = introduced grass mixture
C = native grass-forb mixture
D = introduced grass-forb mixture
E = native grass-forb-shrub mixture
F = native and introduced grass-forb-shrub mixture

Figure 10. Experimental design for the Successional Study on Surface Disturbed Soil.

Table 2. Mixtures seeded during November, 1976, on the Surface Disturbed Successional Study Area.

Common Name	Scientific Name	Seeding Rate PLS (kg/ha)
Mixture A--Native grass mixture		
1. Bearded bluebunch wheatgrass	*Agropyron spicatum*	3.36
2. Rosana western wheatgrass	*A. smithii*	4.48
3. Green needlegrass	*Stipa viridula*	3.36
4. Indian ricegrass	*Oryzopsis hymenoides*	2.24
5. Sodar streambank wheatgrass	*Agropyron riparium*	3.36
Mixture B--Introduced grass mixture		
1. Nordan crested wheatgrass	*Agropyron cristatum*	3.36
2. Luna pubescent wheatgrass	*A. trichophorum*	4.48
3. Vinal Russian wildrye	*Elymus junceus*	3.36
4. Oahe intermediate wheatgrass	*A. intermedium*	4.48
Mixture C--Native grass-forb mixture		
1. Critana thickspike wheatgrass	*Agropyron dasystachyum*	3.36
2. Green needlegrass	*Stipa viridula*	2.24
3. Bearded bluebunch wheatgrass	*Agropyron spicatum*	2.24
4. Indian ricegrass	*Oryzopsis hymenoides*	1.12
5. Sodar streambank wheatgrass	*Agropyron riparium*	2.24
6. Utah sweetvetch	*Hedysarum boreale utahensis*	1.12
7. Emerald crownvetch	*Coronilla varia*	1.12
8. Lewis flax	*Linum lewisii*	1.12
9. Palmer penstemon	*Penstemon palmeri*	1.12
Mixture D--Introduced grass-forb mixture		
1. Vinal Russian wildrye	*Elymus junceus*	3.36
2. Nordan crested wheatgrass	*Agropyron cristatum*	3.36
3. Luna pubescent wheatgrass	*A. trichophorum*	3.36
4. Ladak alfalfa	*Medicago sativa*	1.12
5. Bouncing bet	*Saponaria officinalis*	1.12
6. Small burnet	*Sanguisorba minor*	1.12
7. Lutana cicer milkvetch	*Astragalus cicer*	2.24
Mixture E--Native grass-forb-shrub mixture		
1. Indian ricegrass	*Oryzopsis hymenoides*	2.24
2. Bearded bluebunch wheatgrass	*Agropyron spicatum*	2.24
3. Rosana western wheatgrass	*A. smithii*	4.48
4. Emeral crownvetch	*Coronillia varia*	1.12
5. Utah sweetvetch	*Hedysarum boreale utahensis*	1.12
6. Stansbury cliffrose	*Cowania mexicana stansburiana*	1.12
7. Green ephedra	*Ephedra viridis*	1.12

Table 2.--Continued

Common Name	Scientific Name	Seeding Rate PLS (kg/ha)
8. Fourwing saltbush	*Atriplex canescens*	2.24
9. Winterfat	*Ceratoides lanata*	1.12
Mixture F--Native and introduced grass-forb-shrub mixture		
1. Green needlegrass	*Stipa viridula*	2.24
2. Bearded bluebunch wheatgrass	*Agropyron spicatum*	2.24
3. Nordan crested wheatgrass	*A. cristatum*	2.24
4. Luna pubescent wheatgrass	*A. trichophorum*	2.24
5. Lutana cicer milkvetch	*Astragalus cicer*	1.12
6. Utah sweetvetch	*Hedysarum boreale utahensis*	1.12
7. Stansbury cliffrose	*Cowania mexicana stansburiana*	1.12
8. Green ephedra	*Ephedra viridis*	2.24
9. Winterfat	*Ceratoides lanata*	1.12

shrubs. Sub-subplot treatments included two fertilizer levels and a control as listed below:

1. 112 kg N, 56 kg P per hectare
2. 56 kg N, 28 kg P per hectare
3. Control, 0 kg N, 0 kg P per hectare

Phosphorus, in the form of triple super phosphate (0-46-0) was applied at the above rates to the randomly selected plots and rototilled into the soil at the time of plot construction, but nitrogen fertilizer in the form of ammonium nitrate (32-0-0) was applied following the first growing season, as it was believed the salt effect of the fertilizer might retard growth of seedlings under moisture stress. Subplots were planted in November, 1976, into a rather loose seedbed. This seedbed condition was the result of an unusually dry fall coupled with mechanical disturbance. Planting was followed immediately by hydromulching with 2.2 metric tons/ha of wood fiber applied in a water emulsion.

Vegetation was sampled for emergence in May and June of 1977 as well as establishment in August of 1977. Density and maximum height were recorded by life form (by species when identifiable). Because of the paucity of precipitation and resultant poor growth the biomass and cover measurements were omitted from the first season measurements.

The second growing season allowed the plants to mature enough to be identifiable so the 1978 data includes:

1. Number of plants by species per plot
2. Biomass of plants by species
3. Cover value of plants by species
4. Maximum height of plants by species
5. Number of seed stalks per quadrat by plant species

The winter and spring following planting were extremely dry with less than 7.6 cm of precipitation received on areas adjacent to the study site. This drought continued into the summer of 1977 and strongly influenced the species composition and densities of the established stands. Winter and spring precipitation during the 1977-1978 season was much higher and resulted in very rapid growth of the surviving plants. Field observations and a synopsis of vegetation responses from the 1977 field season were reported in the March 1978 Progress Report.

The results of the preliminary analysis of the density, biomass, and cover of seeded and invading species for the 1978 field season are presented in the following discussion. Reduction and synthesis of the vigor indices and other information among years is now in progress.

Effect of Seed Mixture on Seeded and Invading Species by Life Form

The seed mixture, to a large degree, determines the biomass, cover, and relative numbers of plants in the established vegetation communities. Seed mixture had a pronounced effect on the biomass of the seeded grass component as shown in Figure 11. The introduced grass mixture (Seed Mixture 2) had the highest grass biomass (51 g/¼ m^2). These mixtures all contained introduced grasses, although the amount of grass seed planted in them varied from 15.7 kg/ha in Mixture 2 to a low of 9 kg/ha in Mixture 6. As can be seen, the seeding rate of grass did not appear to be the sole controlling factor upon the grass biomass.

Native seedings had significantly lower grass biomass than the introduced grass mixture and the combination of native and introduced grass-forb-shrub mixture. There was no significant difference in grass biomass between mixtures that were composed of only native species

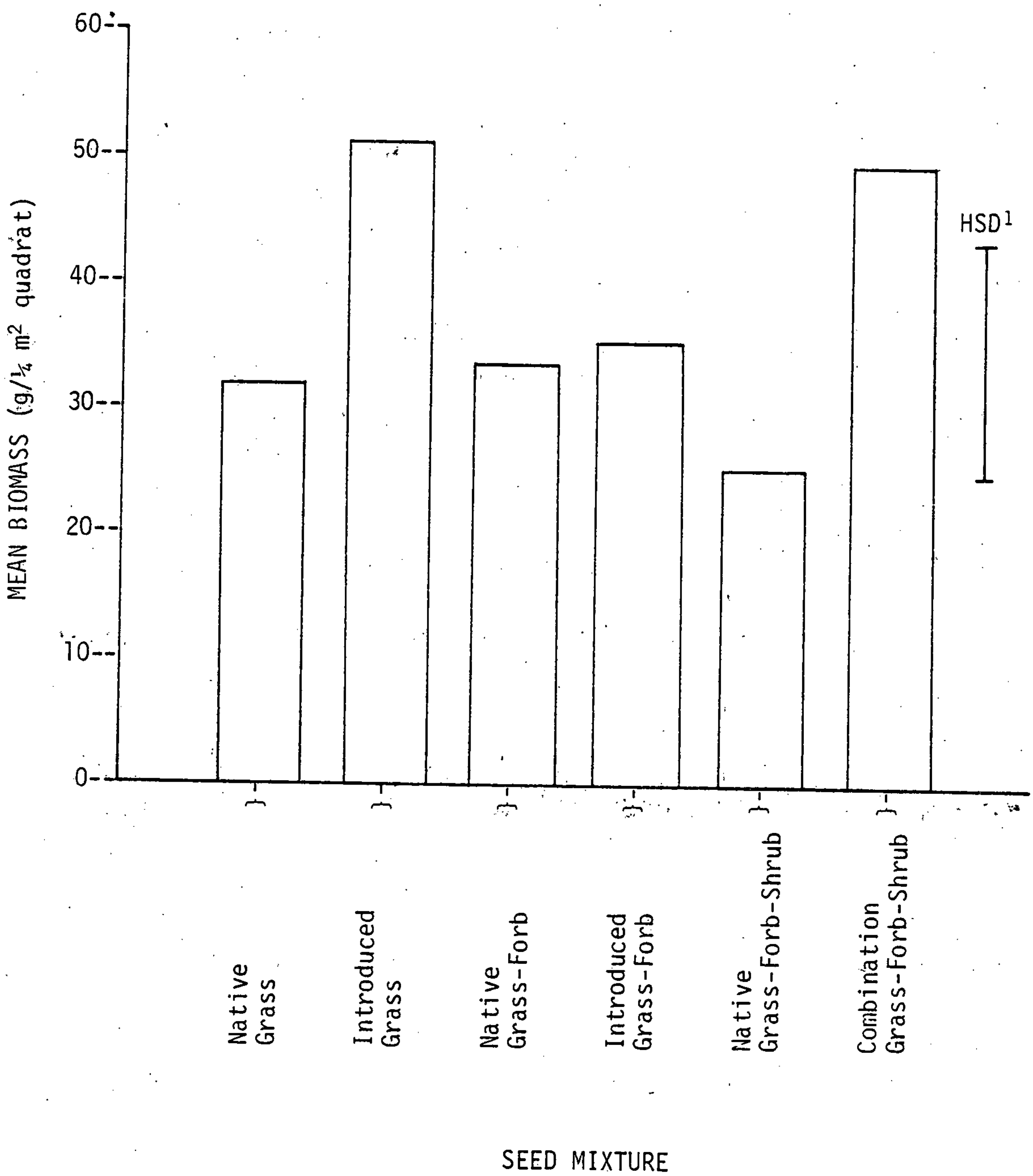


Figure 11. Biomass of seeded grasses for the Surface Disturbed Successional Plots.

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[1]Tukey's honestly significant difference at $P = .05$.

(Mixtures 1, 3, and 5). The biomass of invading grasses was highest in the seedings of native species and lowest in the introduced mixtures (Figure 12).

Seed mixture also had a significant effect upon the biomass of seeded forbs (Figure 13). The introduced grass-forb mixture had the highest biomass. This expression can be attributed to the inclusion of Ladak alfalfa (*Medicago sativa*), bouncing bet (*Saponaria officinalis*), and small burnet (*Sanguisorba minor*) in the mixture. These forbs have the capability to establish and grow even though they must compete with introduced grasses. The biomass of invading forbs (Figure 14) responded to the seed mixture in the same manner as the invading grasses previously discussed; that is, with the highest forb biomass in the native mixtures and lowest in mixtures that included introduced species.

The seed mixture planted had a highly significant effect upon the biomass of seeded shrubs (Figure 15). This effect was the result of two factors: first, the presence of fourwing saltbush (*Atriplex canescens*) in the native grass-forb-shrub mixture (Seed Mixture 5) and second, competition from introduced grasses in the combination native and introduced grass-forb-shrub seed mixture (Seed Mixture 6). Observations by field personnel suggest that fourwing saltbush can produce much higher biomass than the other shrub species planted. These plants may be .5 m tall and weigh 300 g. This capacity for higher biomass would account for much of the difference between seed mixtures. It was also observed, however, that the introduced grasses reduced the size and vigor of winterfat (*Ceratoides lanata*), which made up a major portion of the shrub community on both plantings that included shrubs (Seed Mixtures 5 and 6).

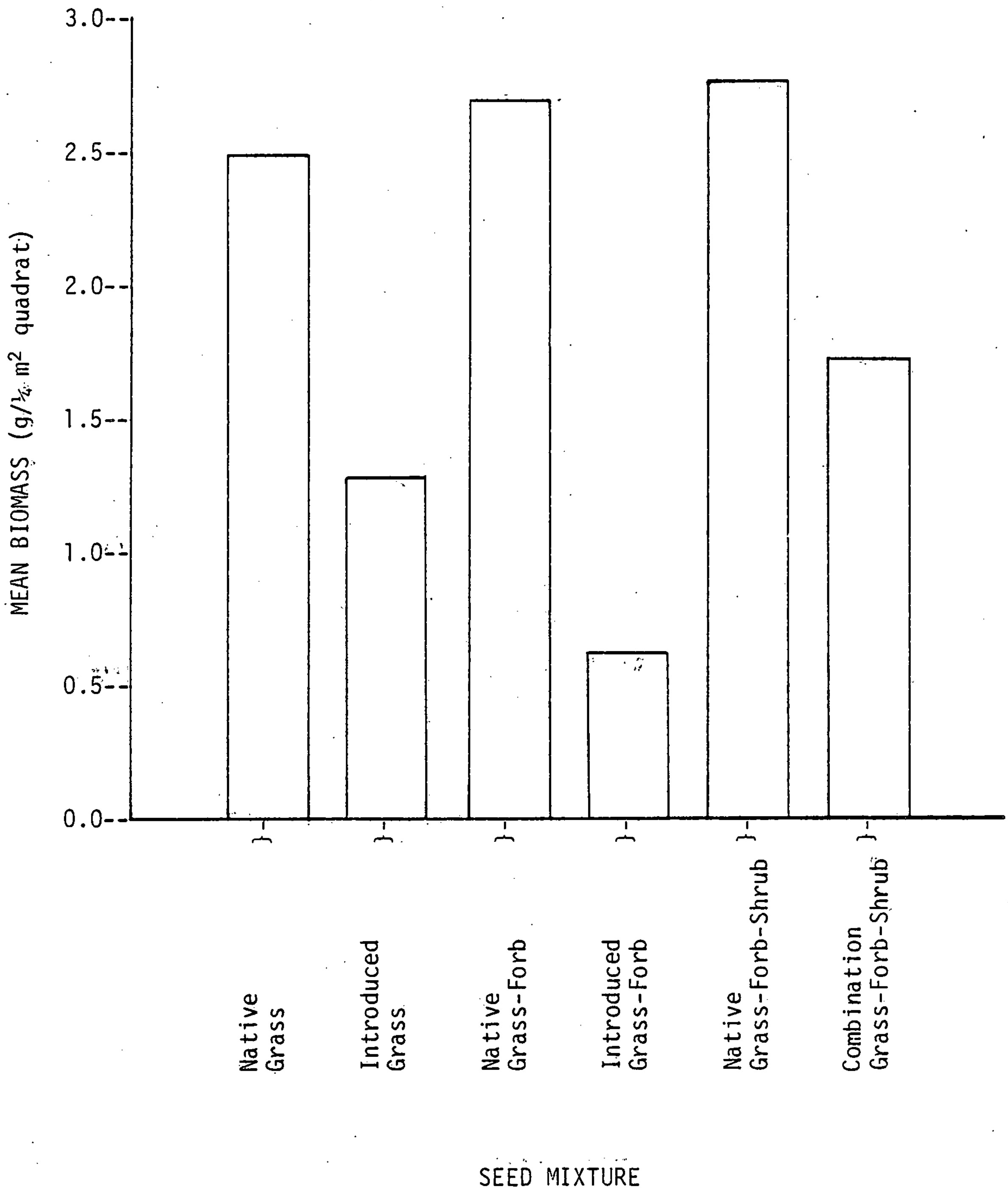

Figure 12. Biomass of invading grasses plotted against seed mixture on the Surface Disturbed Successional Plot.

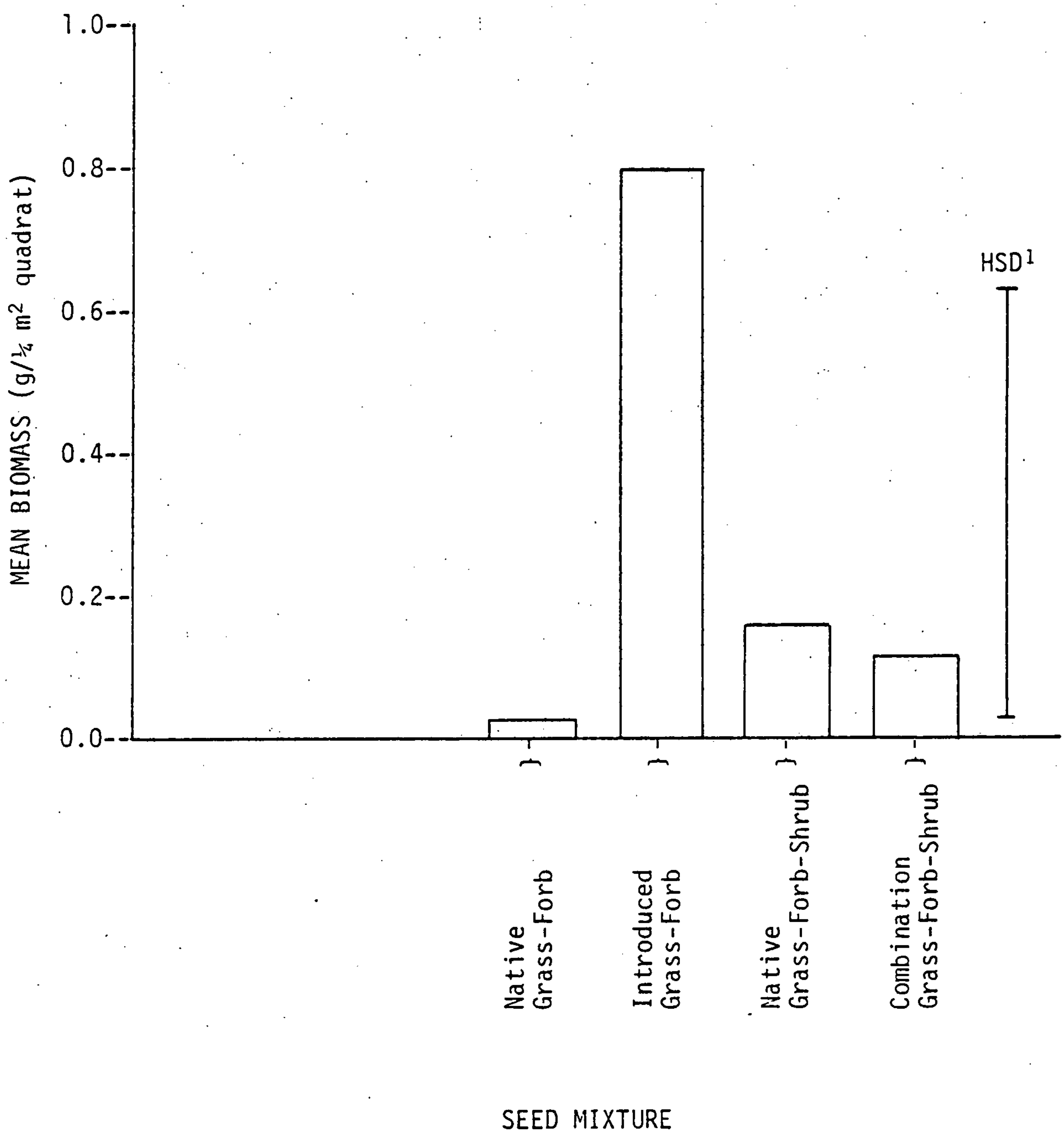

Figure 13. Biomass of seeded forbs for the Surface Disturbed Successional Plot.

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[1]Tukey's honestly significant difference at $P = .05$.

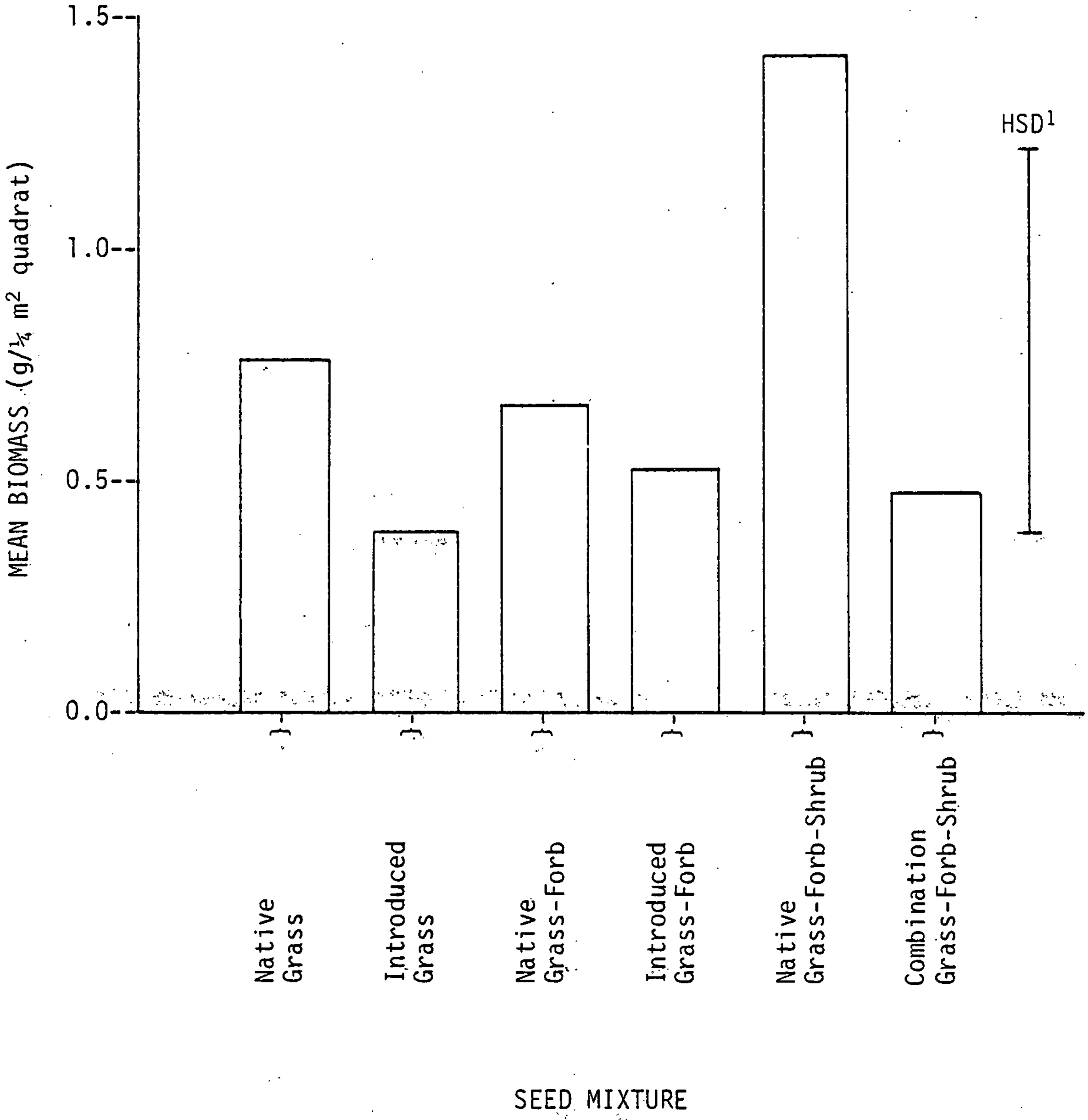

Figure 14. Biomass of invading forbs plotted against seed mixture for the Surface Disturbed Successional Plot.

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[1]Tukey's honestly significant difference at P = .05.

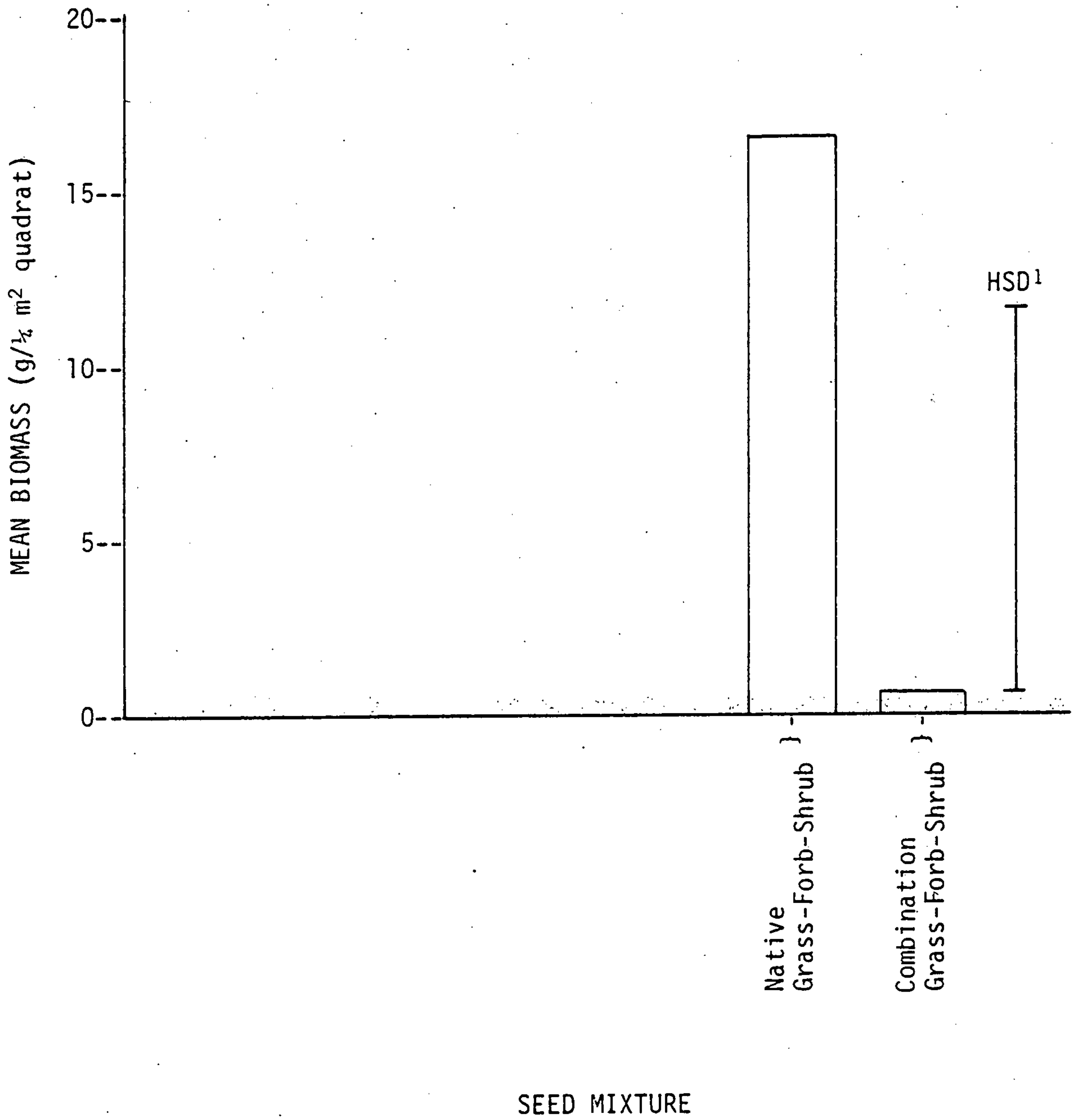

Figure 15. Biomass of seeded shrubs for the Surface Disturbed Successional Plot.

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[1] Tukey's honestly significant difference at P = .05.

There was no significant effect of seed mixture on invading shrub biomass. The principle invading shrubs were Douglas rabbitbrush (*Chrysothamnus viscidiflorus*) and Broom snakeweed (*Gutierrezia sarothrae*).

The mixture planted had no significant effect on the cover of seeded grasses, but it did affect the cover of invading grasses. Cheatgrass brome (*Bromus tectorum*) was the most common invading grass. This grass is an annual that can form very dense stands on disturbed lands and may retard the establishment of plants characteristic of advanced successional stages. Other invading grasses were needleandthread (*Stipa comata*), prairie junegrass (*Koeleria cristata*), bottlebrush squirreltail (*Sitanion hystrix*), and Indian ricegrass. The highest cover values for the invading grasses as a group were found in the native grass-forb-shrub seed mixture (1.58 percent), native grass mixture (1.40 percent), and native grass-forb mixture (1.33 percent). The combination seed mixture of native and introduced grasses, forbs, and shrubs had a mean invading grass cover of 1.31 percent. Introduced grass and introduced grass-forb mixtures had mean invading grass covers of 0.70 percent and 0.88 percent, respectively. The cover values for the invading grasses followed the same general pattern as was seen in the biomass of invading grasses, i.e., higher values in the native mixtures.

The mixture seeded also affected the cover of seeded forbs (Figure 16), which was higher with introduced species. Seeded shrubs had higher cover values in the native mixture because of the presence of the aggressive fourwing saltbush in the mixture (Figure 17).

The densities of seeded grasses, forbs, and shrubs were all affected by the seed mixture, as might be expected. However, the

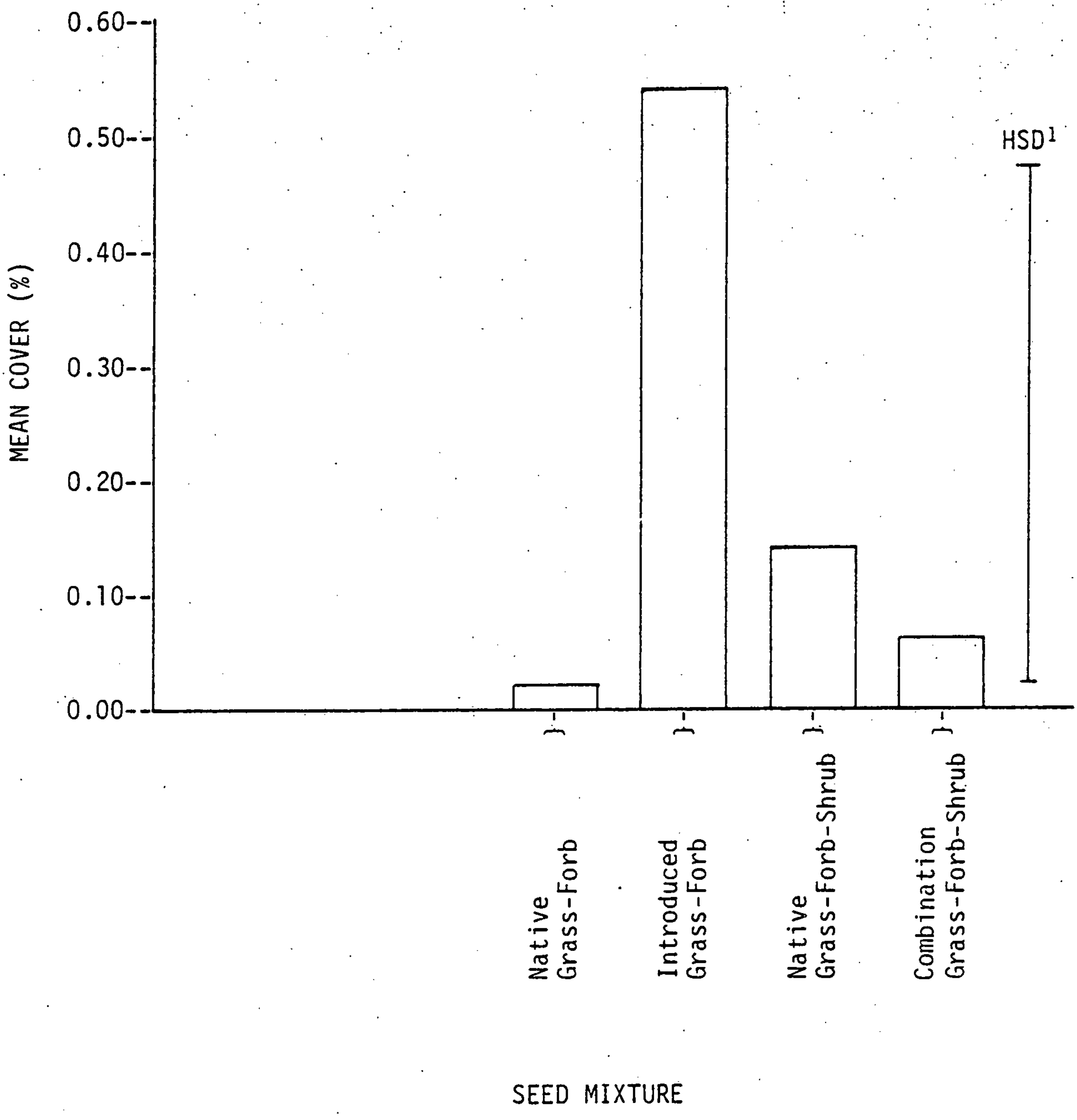

Figure 16. Cover of seeded forbs for each seed mixture for the Surface Disturbed Successional Plot.

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[1]Tukey's honestly significant difference at P = .05.

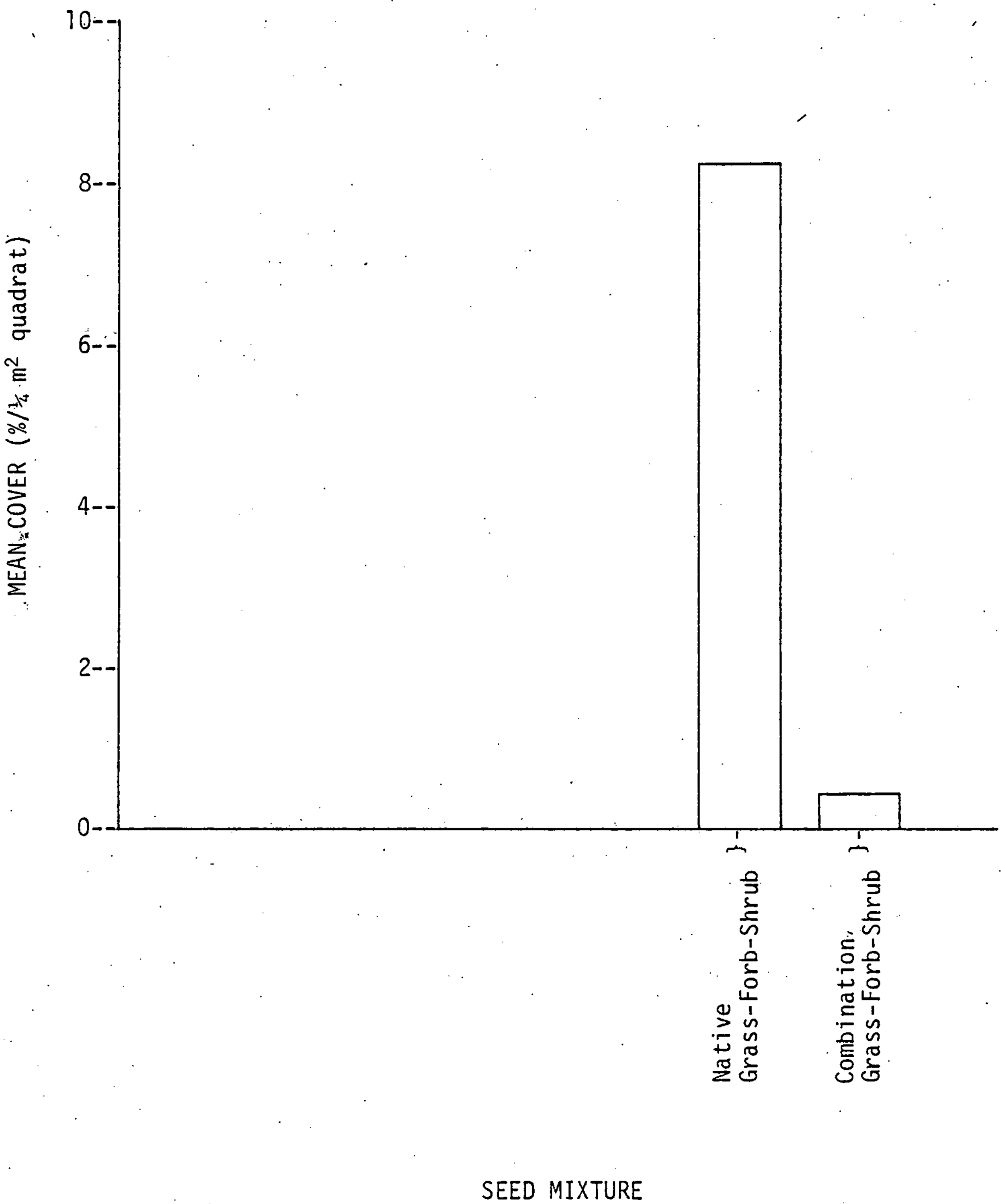

Figure 17. Cover of seeded shrubs for those seed mixtures which were planted on the Surface Disturbed Successional Plot.

densities of invading grasses, forbs, and shrubs were not significantly different among seed mixtures.

Invading plants are present in all sub-subplots, but their capacity to grow (as measured by biomass and cover values) was reduced by competition from the planted introduced species.

Effect of Fertilization on Seeded and Invading Species by Life Form

Fertilizer additions affected both the biomass and cover of seeded grasses. The application of fertilizer increased biomass of seeded grasses significantly from 31 to 42 g/¼ m^2 (Figure 18). Cover values for the seeded grasses also increased significantly with fertilizer (Figure 19). The mean cover value was 9 percent with no fertilizer but was 11.8 percent and 12.3 percent for Fertility Treatments 2 and 3, respectively.

The biomass and cover of invading grasses was not significantly changed by the fertilizer treatment, nor was the biomass of seeded forbs significantly affected (P=.05) by the addition of fertilizer. However, fertilizer had a significant negative effect on the cover of seeded forbs (Figure 20). This response is not due to the action of the fertilizer directly but rather appears to be a result of the effect of fertilizer on the grasses. Grasses respond to fertilization more rapidly than do forbs, which results in increased competitive stress for the forb component. The cover of invading forbs was not significantly affected by fertilizer.

Fertilizer had no significant effect on the biomass or cover of seeded shrubs or on the biomass or cover of invading shrubs.

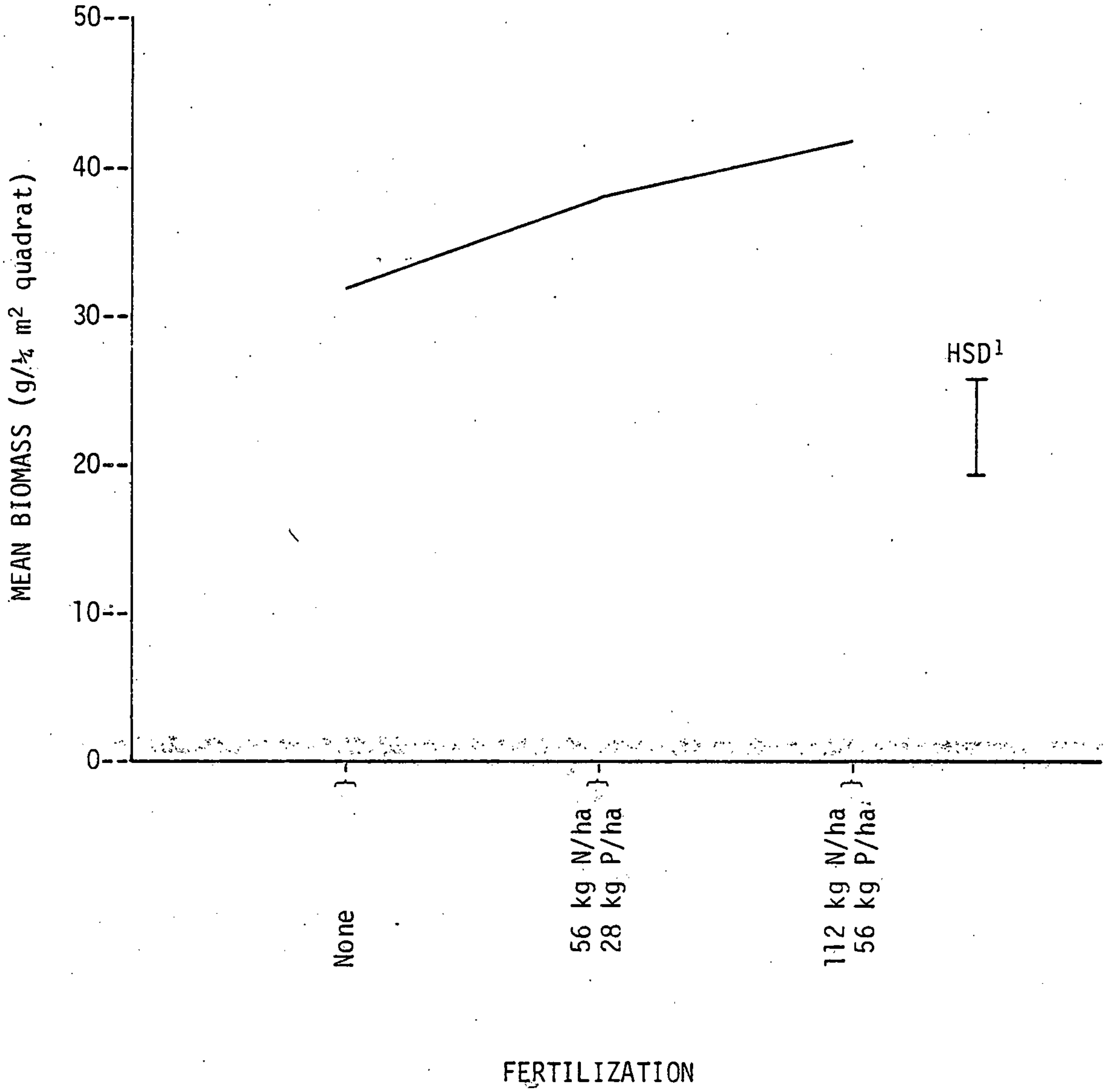

Figure 18. Biomass of seeded grasses plotted against fertilizer additions for the Surface Disturbed Successional Plot.

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[1]Tukey's honestly significant difference at $P = .05$.

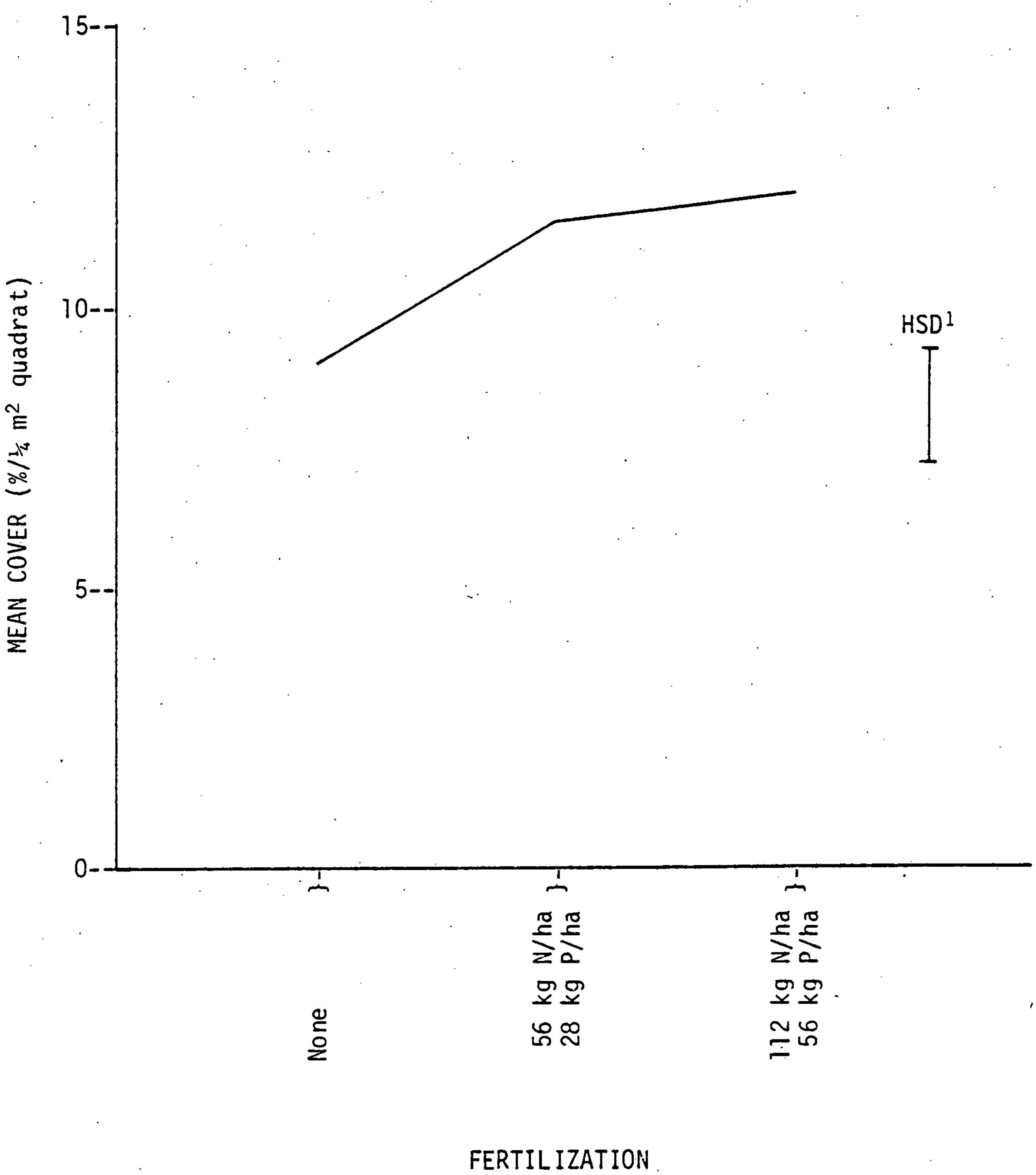

Figure 19. Cover of seeded grasses plotted against fertilizer additions for the Shallowly Disturbed Successional Plot.

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[1]Tukey's honestly significant difference at P = .05.

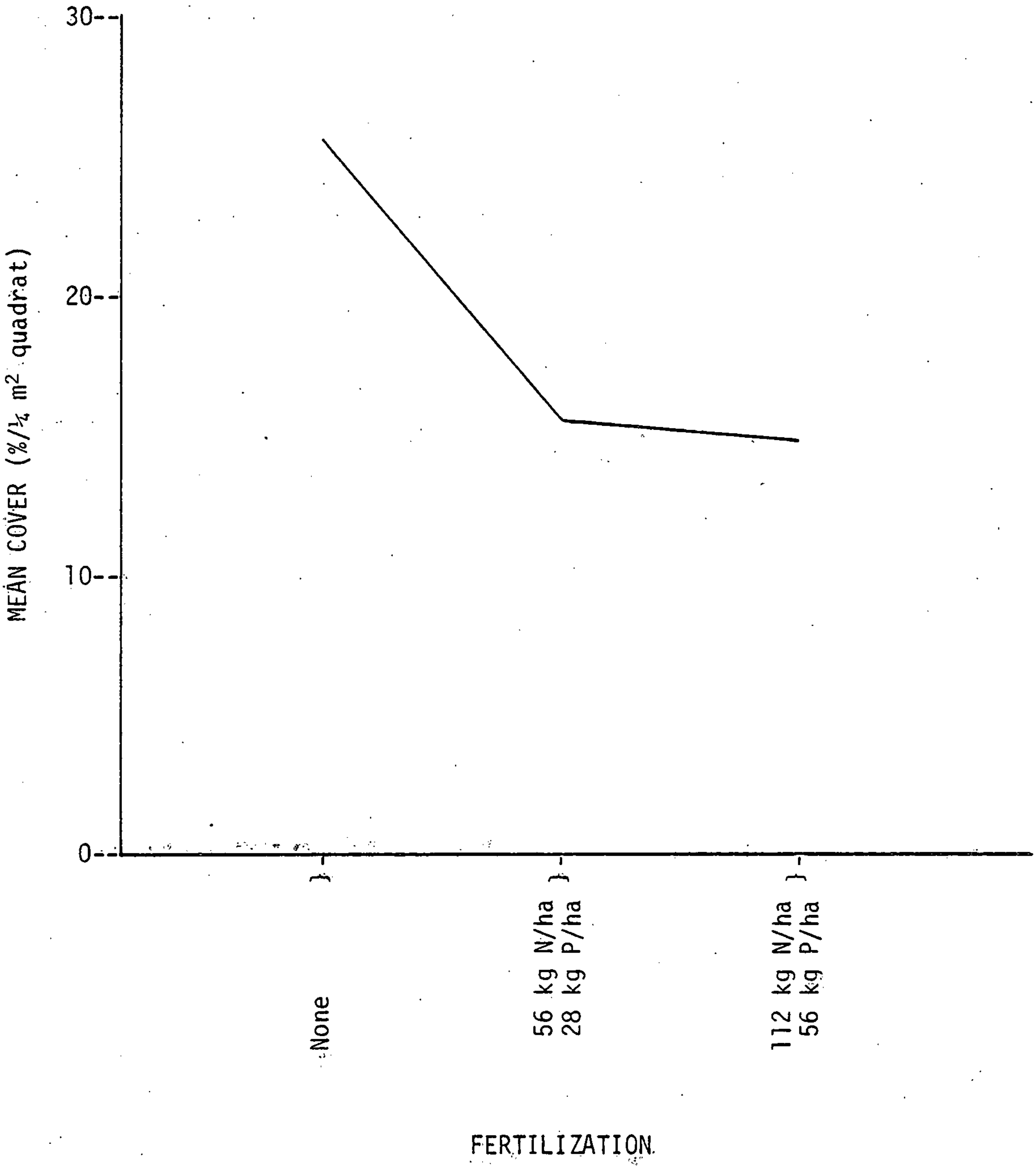

Figure 20. Cover of seeded forbs plotted against fertilizer additions for the Shallowly Disturbed Successional Plot.

The density of seeded grasses had a significant interaction among fertility, seed mixture, and mulch (Figure 21). This effect was largely a result of discrepancy among the various relationships. The interaction effect of fertilizer was confined primarily to the native grass-forb-shrub mixture. For all other mixtures there was no significant effect of fertilizer treatment on the density of seeded grasses. There were no significant fertilizer effects on the density of invading grasses.

Fertilizer additions had no significant effect on the density of seeded or invading forbs.

The density of seeded shrubs demonstrated an interaction with fertilizer, mulch, and seed mixture; but, as with the density of grasses, this effect was confined to the response of the native grass-forb-shrub seed mixture (Figure 22). With no mulch, fertilization reduced the number of shrubs; however, with mulch, fertilization increased the number of shrubs. The reason for this is unknown at this time.

Effect of Mulch on Seeded and Invading Species by Life Form

The mulching treatment was not randomly applied to each plot; therefore, the effects have to be determined by examination of mulch interactions from the analysis of variance as a split block (Appendix A, Tables 4-8). Mulching had no significant effect on the biomass of seeded or invading grasses, forbs, or shrubs. Cover values for grasses or shrubs were unaffected by mulch; however, the seeded forbs had a mulch-seed mixture interaction that was significant at the $P = .10$ level. There is no clear trend to explain this information (Figure 23). Mulch treatment had no significant effect on the cover of invading forbs.

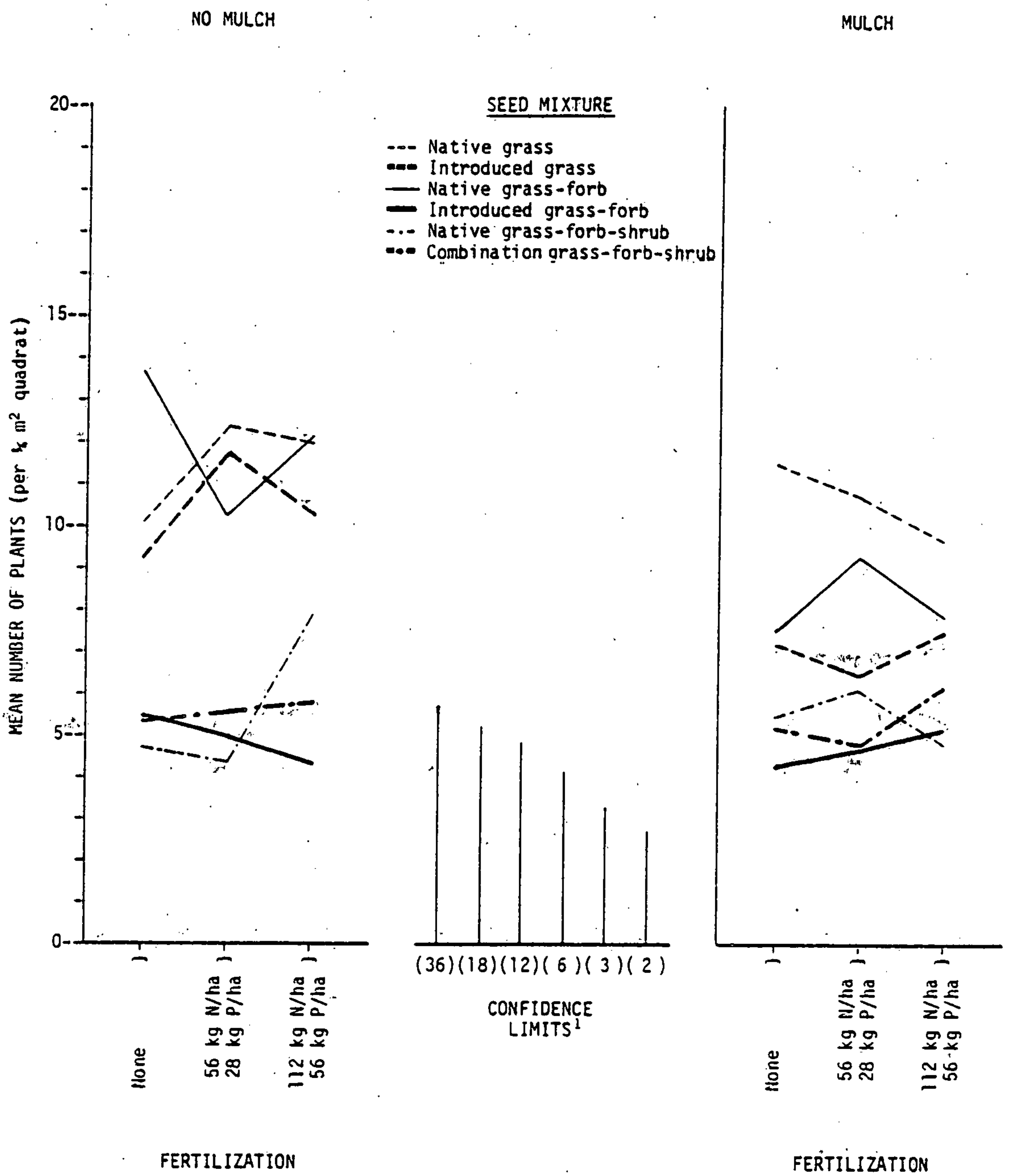

Figure 21. Density of seeded grasses plotted against treatment for the Surface Disturbed Successional Plots.

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[1] The honest significant difference procedure or Tukey's Q-test was used to determine significant differences between means in a significant main effect or interaction. Confidence intervals are shown above the number of means to be compared. Within interactions a group of means representing all levels of one treatment and at one level of the other treatments can be compared using the confidence interval above the number of means in the group.

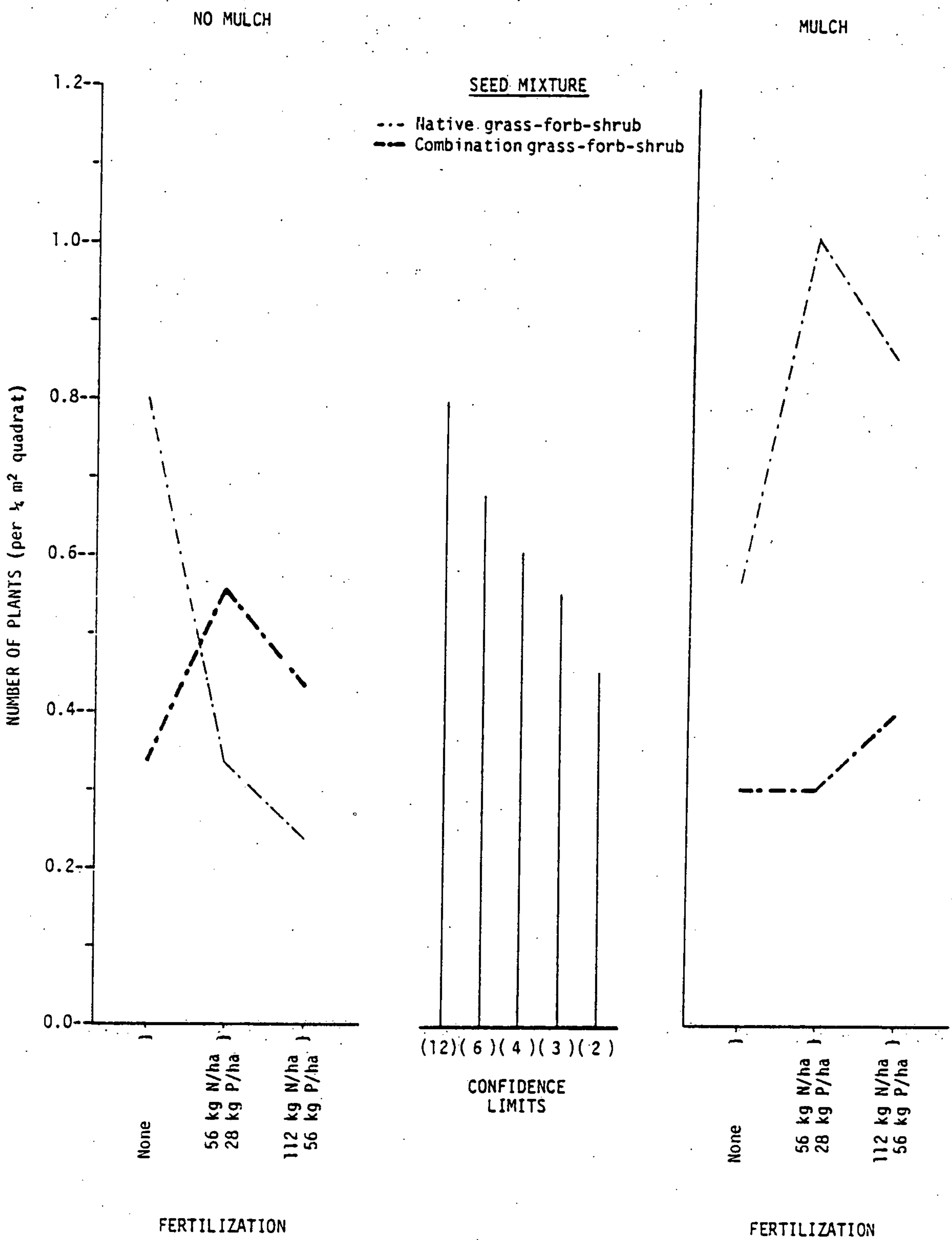

Figure 22. Density of seeded shrubs plotted against treatment for the Surface Disturbed Successional Plots.

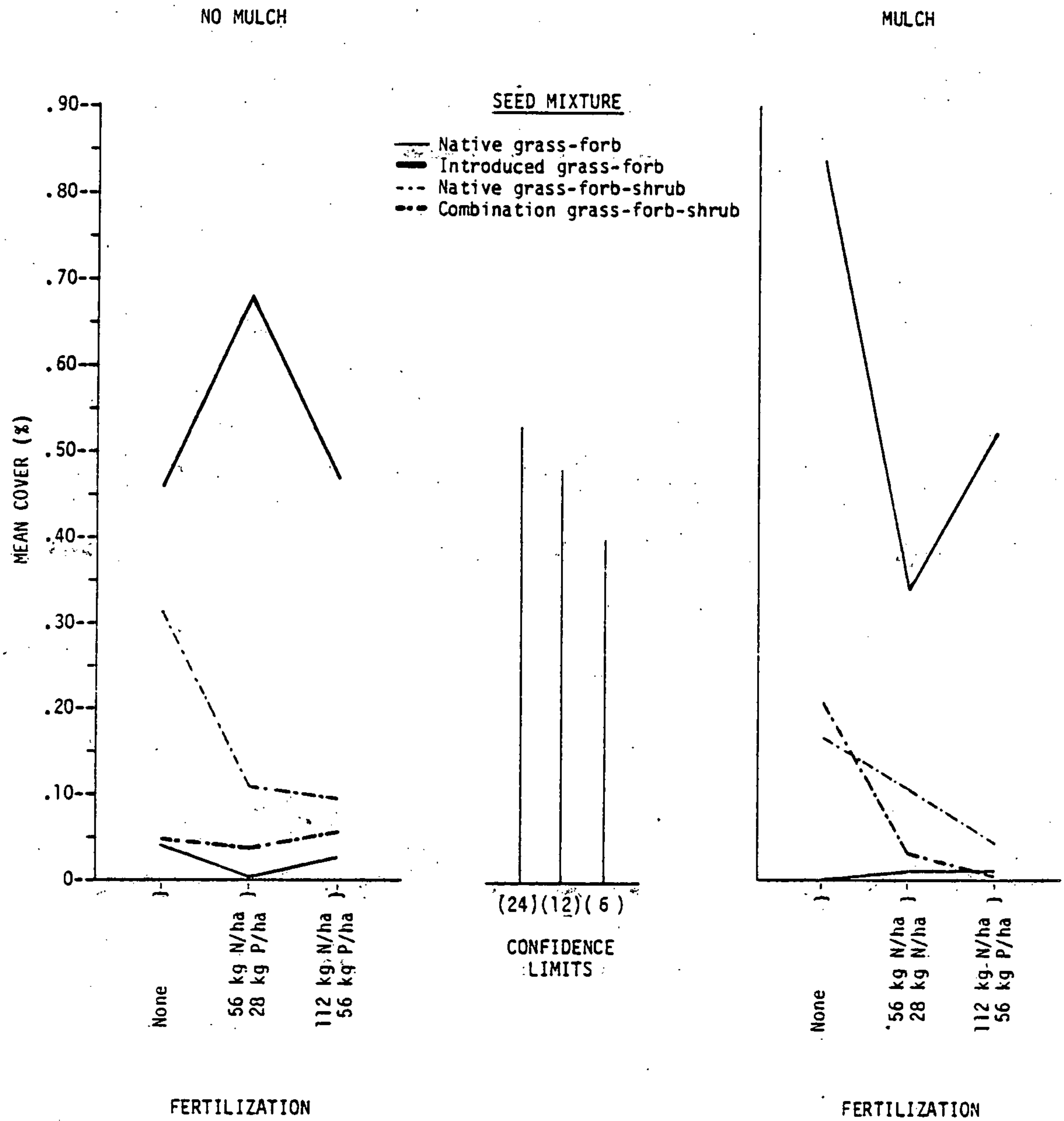

Figure 23. Cover of seeded forbs for each treatment on the Surface Disturbed Successional Plots.

The density values for seeded grasses were affected by a mulch-fertilizer-seed mixture interaction (Figure 21). This information indicates that the mulch may retard the establishment of grasses in both the native and introduced grass and grass-forb mixtures. There was no significant effect of mulch on invading grass densities or on the density of seeded or invading forbs.

Shrub densities responded to mulch in an interaction with fertilizer level applied and seed mixture planted. Mulch appears to enhance the number of shrubs that establish in the native mixture when fertilized. This appears to be the combined result of protection of the seedbed by mulch and stimulation of the shrubs by fertilization.

The mulching treatment had little beneficial effect on the overall stand of seeded species or the amount of invasion that occurred.

Conclusions

The species mixture planted had a pronounced effect upon the established plant communities. Introduced grasses tended to have higher biomass than the native grasses. Also, introduced grasses reduced the biomass of invading grasses. Introduced forbs, likewise, had higher biomass than the native forbs, and the invading forbs tended to have higher biomass when competing with the native grasses than with those mixtures that included introduced grasses. Fourwing saltbush increased the biomass of the shrub component when it was included in the seed mixture. Other shrubs were observed to be retarded by the presence of introduced grasses.

The mixture planted had no significant effect on the cover of seeded grasses, but invading grass cover tended to be less on subplots containing introduced grasses than on those subplots that contained native grasses. The introduced forbs had higher cover values than the native forbs. Shrub cover was higher when fourwing saltbush was included in the species mixture seeded.

The densities of grasses, forbs, and shrubs were affected by the mixture planted, as would be expected; but the densities of invading grasses, forbs, and shrubs were not significantly different between seed mixtures.

Fertilizer additions increased the biomass and cover of seeded grasses and resulted in a decrease in the cover of seeded forbs.

Mulching with wood fiber hydromulch had little beneficial effect on the established stands.

Species reaction to developmental successional patterns will become more important in the next few years as the establishment of closed communities becomes more pronounced among treatments. The spatial mix of plants is expected to change rather markedly from year to year as competition for the environmental resources becomes keener among individual species. Occupancy and dynamics in successional expressions that utlimately lead to dominance will furnish rational conclusions for successful revegetation of disturbances brought about by oil shale development.

Successional Study on Annual Disturbance Plots

A set of specifically disturbed plots was first established in 1976 and 1977. These plots have been sampled once each year following the summer of their construction.

The purpose of these plots is to provide a basis for monitoring the patterns of natural plant invasion on disturbed, nonseeded areas. Plots established yearly are necessary for an adequate evaluation of the effects of climate and other modulating annual influences on the invasion process. The information assembled can be used to compare and contrast the invasion progress on the other successional studies being conducted at the Intensive Study Site.

The experimental design of the plots is shown in Figure 24. Four subplots, each measuring 6x8 m, were separated by 1.5 m buffer zones. Each subplot was then randomly assigned one of the following disturbance treatments.

Treatment 1: Scrape vegetation off while leaving as much topsoil as possible.

Treatment 2: Scrape vegetation off and rip subsoil to a depth of 30.0 cm.

Treatment 3: Remove topsoil and subsoil to a depth of 1.0 m. Mix the soils together and replace in the area from which it was remove.

Treatment 4: Remove 1.0 m of topsoil and subsoil and stockpile. Remove an additional 1.0 m layer of subsoil and stockpile. Replace the material in a reverse order with the final material removed placed on the surface.

In 1978 ten permanent 0.25 m^2 quadrats were randomly positioned within each of the 1976 and 1977 subplots. The plots were sampled to record (by plant species) values of density, cover, and aboveground biomass. The data was analyzed statistically using a two-way

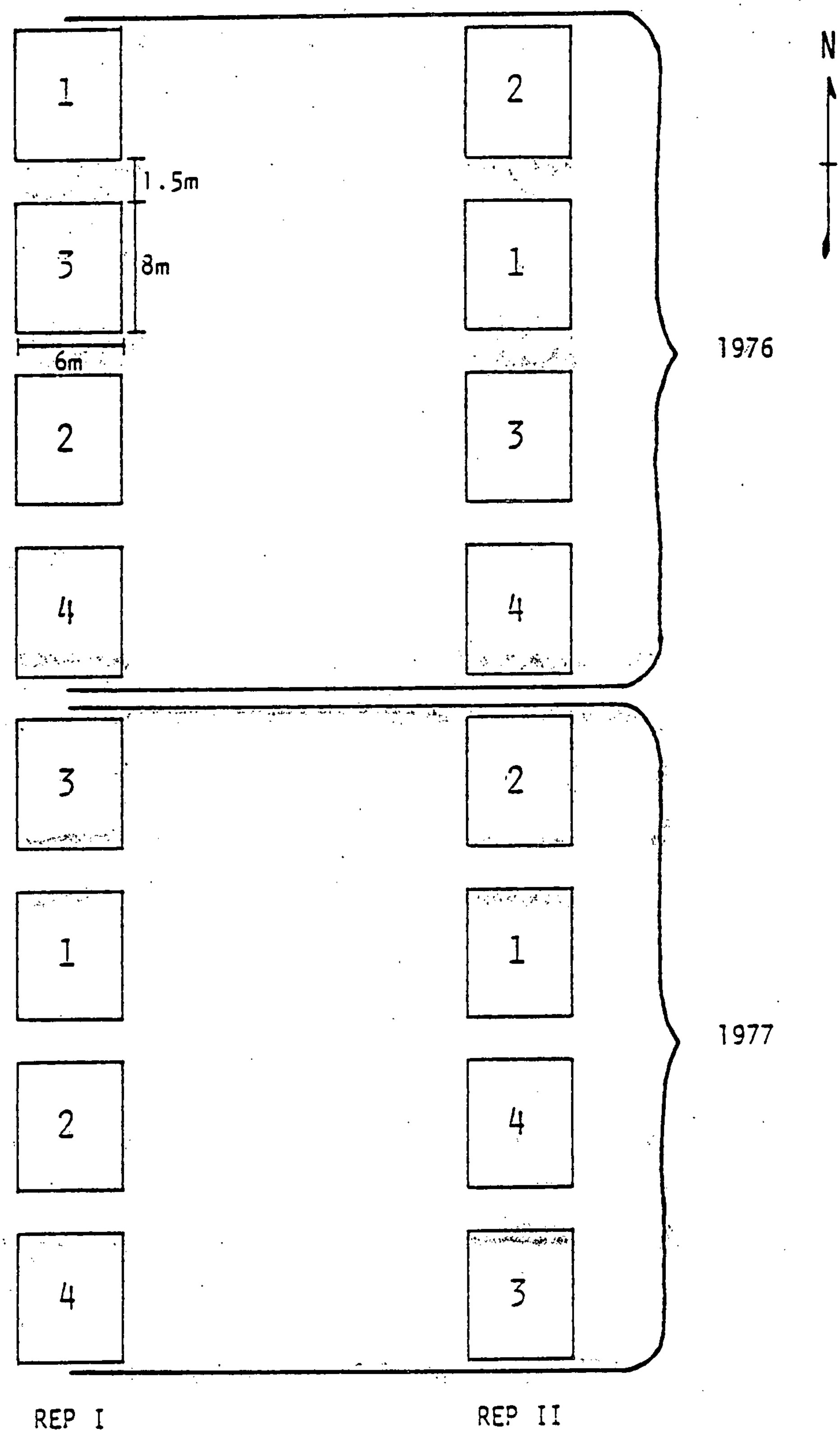

Figure 24. Experimental design for the Annual Disturbance Plot, 1976, 1977.

analysis of variance. When significance was encountered ($P<.05$), a Tukey's Q-test was used to test differences between means.

Results--1976 Plots

Mean density, biomass, and cover values for the plots constructed in 1976 are reported by life form and by plant species in Tables 3-5. Only in forb density was a significant difference found (Table 3). The 16.80 and 7.40 forbs m^{-2} on Treatments 1 and 2, respectively, were significantly different than the 1.20 and 1.00 forbs m^{-2} on Treatments 3 and 4, respectively. Prairie junegrass and scarlet globemallow (*Sphaeralcea coccinea*) on Treatment 1 demonstrated the highest grass and forb density with 23.00 and 8.40 plants m^{-2}, respectively. Indian ricegrass and scarlet globemallow established successfully on all the treatments.

Total plant density, biomass, and cover were the greatest on Treatment 1. Grasses on Treatment 1 produced the highest density and cover of any other life form with values of 56.20 grasses m^{-2} for density and 50.60 percent for cover. The highest mean biomass was recorded for grasses on Treatment 1 and for forbs on Treatments 1 and 3.

Total plant density decreased with the increase in disturbance. Mean total plant biomass and cover, however, decreased in order from Treatment 1, 3, 2, and 4. Forbs were primarily responsible for this order among disturbance treatments.

Shrubs demonstrated essentially negligible establishment on all treatments. Only the few shrubs on Treatment 3 exhibited meaningful biomass and cover.

At a glance, some means between treatments in Table 3-5 appear to be significantly different. However, there were extreme variations

Table 3. Mean number of plant species and life forms per m^2 for each treatment in the 1976 Annual Disturbance Plot sampled in 1978.

Life Form/Species	Treatments[1]			
	1	2	3	4
Grasses				
Koeleria cristata	23.00	1.20	0.20	--
Agropyron dasystachyum	4.00	0.80	--	--
A. riparium	2.00	2.20	0.40	--
Agropyron spp.	17.00	2.60	--	--
Oryzopsis hymenoides	1.20	0.60	0.40	0.60
Stipa comata	9.00	0.40	--	--
TOTAL	56.20[a]	7.80[a]	1.00[a]	0.60[a]
Forbs				
Salsola kali	1.40	0.20	0.80	--
Sphaeralcea coccinea	8.40	4.00	0.20	0.60
Chenopodium spp.	0.20	--	--	--
Phlox hoodii	3.40	1.60	--	--
Trifolium gymnocarpon	1.80	1.20	0.20	--
Cryptantha sericea	0.40	--	--	--
Descurania pinnata	0.20	0.20	--	0.20
Astragalus spatulatus	0.80	--	--	--
Haplopappus nutallii	0.20	--	--	0.20
Townsendia hookeri	--	0.20	--	--
TOTAL	16.80[a]	7.40[a]	1.20[b]	1.00[b]
Shrubs				
Chrysothamnus viscidiflorus	0.20	--	--	--
C. nauseosus	--	--	--	0.20
Gutierrezia sarothrae	--	0.20	0.20	--
TOTAL	0.20[a]	0.20[a]	0.20[a]	0.20[a]

Table 3.--Continued

Life Form/Species	Treatments[1]			
	1	2	3	4
Grasses + Forbs + Shrubs				
TOTAL	73.20[a]	15.40[a]	2.40[a]	1.80[a]

[1]Treatment 1 = Surface scraped with caterpillar blade; Treatment 2 = Surface scraped and ripped to 30.0 cm; Treatment 3 = Surface scraped, soil removed to a 1.0 m depth, mixed, and replaced; Treatment 4 = Surface scraped, 2.0 m of soil removed, and replaced in reverse order.

[a,b]Means in the same row bearing different superscript letters are significantly different ($P<.05$).

Table 4. Mean biomass (g m^{-2}) of plant species and life forms for each treatment in the 1976 Annual Disturbance Plot sampled in 1978.

Life Form/Species	Treatments[1]			
	1	2	3	4
Grasses				
Koeleria cristata	18.25	2.60	0.05	--
Agropyron dasystachyum	11.30	1.00	--	--
A. riparium	4.30	2.25	0.20	--
Agropyron spp.	7.50	10.20	--	--
Oryzopsis hymenoides	0.90	1.40	0.10	0.15
Stipa comata	8.85	1.40	--	--
TOTAL	51.20[a]	18.85[a]	0.35[a]	0.15[a]
Forbs				
Salsola kali	52.85	0.20	57.40	--
Sphaeralcia coccinea	10.10	8.45	1.00	2.40
Chenopodium spp.	0.05	--	--	--
Phlox hoodii	0.85	0.30	--	--
Trifolium gymnocarpon	0.45	0.35	0.05	--
Cryptantha sericea	0.10	--	--	--
Descurania pinnata	0.05	0.20	--	0.20
Astragalus spatulatus	0.05	--	--	--
Haplopappus nuttallii	0.20	--	--	0.60
Townsendia hookeri	--	0.05	--	--
TOTAL	64.50[a]	9.55[a]	58.45[a]	3.20[a]
Shrubs				
Chrysothamnus viscidiflorus	0.05	--	--	--
C. nauseosus	--	--	--	0.80
Gutierrizia sarothrae	--	0.20	4.00	--
TOTAL	0.05	0.20	4.00	0.80
Grasses + Forbs + Shrubs				
TOTAL	115.75[a]	28.60[a]	62.80[a]	4.15[a]

[1]See Table 3 for description of treatments.

[a]Means in the same row bearing different superscript letters are significantly different (P<0.05).

Table 5. Mean percentage cover of plant species and life forms per m^2 for each treatment in the 1976 Annual Disturbance Plot sampled in 1978.

Life Form/Species	Treatment[1] 1	2	3	4
Grasses				
Koeleria cristata	30.80	1.00	0.05	--
Agropyron dasystachyum	4.45	0.60	--	--
A. riparium	1.70	2.25	0.05	--
Agropyron spp.	3.05	0.70	--	--
Oryzopsis hymenoides	0.35	1.00	0.10	0.15
Stipa comata	10.25	1.20	--	--
TOTAL	50.60[a]	6.75[a]	0.20[a]	0.15[a]
Forbs				
Salsola kali	9.25	0.05	20.25	--
Sphaeralcia coccinea	7.10	7.65	0.40	1.60
Chenopodium spp.	0.05	--	--	--
Phlox hoodii	1.70	0.50	--	--
Trifolium gymnocarpon	0.60	0.35	0.05	--
Cryptantha sericea	0.10	--	--	--
Descurania pinnata	0.05	0.70	--	0.05
Astragalus spatulatus	0.05	--	--	--
Haplopappus nuttallii	0.05	--	--	0.20
Townsendia hookeri	--	0.05	--	--
TOTAL	18.90[a]	9.30[a]	20.70[a]	1.85[a]
Shrubs				
Chrysothamnus viscidiflorus	0.05	--	--	--
C. nauseosus	--	--	--	0.40
Gutierrizia sarothrae	--	0.05	3.20	--
TOTAL	0.05[a]	0.05[a]	3.20[a]	0.40[a]
Grasses + Forbs + Shrubs				
TOTAL	69.55[a]	16.10[a]	24.10[a]	2.40[a]

[1]See Table 3 for description of treatments.

[a]Means in the same row bearing different superscript letters are significantly different ($P<0.05$).

among replications within these treatments which were too large for the means of the treatment values to be significant in the analysis of variance.

Plant species which were present in the 1976 plots but not present within the sampled quadrats included: bottlebrush squirreltail, bluebur stickseed (*Lappula redowskii*), phacelia (*Phacelia* spp.), Utah sweetvetch, Lewis flax (*Linum lewisi*), fleabane (*Eriogonum* spp.), meadow milkvetch (*Astragalus diversifolius*), penstemon (*Penstemon* spp.), and winterfat.

In comparson of 1977 and 1978 (Tables 3 and 6), grasses and forbs exhibited an apparent increase in density in 1978. Treatments 1 and 2 showed the largest density increase with forbs making the greatest increase over grasses and shrubs. Grasses, however, continued to exhibit the highest mean densities. This increase in density may be attributed to the favorable spring moisture conditions provided by winter snowfall. Shrubs remained the least established of the life forms. Treatments 3 and 4 continued to have negligible plant establishment for all life forms.

Results--1977 Plots

Mean density, biomass, and cover values for the 1978 sampling of the 1977 plots are represented in Table 7, 8, and 9. As with the 1976 plots, significant differences appeared only in plant density (Table 6). The number of grasses on Treatment 1 were significantly greater than on the other treatments. Also, grasses, forbs, and shrubs together were of a significantly higher density on Treatment 1 than on Treatments 2-4.

Grasses displayed a higher density, biomass, and cover on Treatment 1 than on the other treatments. Forb density and cover were also high on Treatments 1 and 2 with forb biomass being the highest on Treatment 2.

Table 6. Mean number of grasses, forbs, shrubs, and total vegetation per m^2 for each treatment in the 1976 Annual Disturbance Plot sampled in 1977.

Treatment[1]	Grasses	Forbs	Shrubs	G + F + S[2]
1	30.20[a]	10.00[a]	0.00[a]	40.20[a]
2	0.80[b]	1.60[b]	0.00[a]	2.40[b]
3	0.20[b]	0.00[b]	0.00[a]	0.20[b]
4	0.00[b]	0.00[b]	0.00[a]	0.00[b]

[1]See Table 3 for description of treatments.

[2]G = grasses; F = forbs; S = shrubs.

[a,b]Means in the same column bearing different superscript letters are significantly different ($P<.05$).

Table 7. Mean number of plant species and life forms per 1.0 m² for each treatment in the 1977 Annual Disturbance Plot sampled in 1978.

Life Form/Species	Treatment[1]			
	1	2	3	4
Grasses				
Oryzopsis hymenoides	3.80	0.60	--	--
Agropyron smithii	2.00	--	--	--
unknown grasses	--	--	0.20	--
TOTAL	5.80[a]	0.60[b]	0.20[b]	0.00[b]
Forbs				
Astragalus spatulatus	0.20	--	0.20	--
Phlox hoodii	0.20	0.20	--	--
Salsola kali	0.20	0.40	--	0.20
unknown forbs	1.00	--	--	--
Descurania pinnata	0.40	--	--	--
Trifolium gymnocarpon	0.60	0.60	--	--
Chenopodium fremontii	0.20	--	--	--
C. album	--	0.20	--	--
Astragalus purshii	0.20	--	--	--
TOTAL	3.00[a]	1.40[a]	0.20[a]	0.20[a]
Shrubs				
TOTAL	0.00[a]	0.00[a]	0.00[a]	0.00[a]
Grasses + Forbs + Shrubs				
TOTAL	8.80[a]	2.00[b]	0.40[b]	0.20[b]

[1]See Table 3 for description of treatments.

[a,b]Means in the same row bearing different superscript letters are significantly different ($P<0.05$).

Table 8. Mean biomass (g m^{-2}) of plant species and life forms for each treatment in the 1977 Annual Disturbance Plot sampled in 1978.

Life Form/Species	Treatment[1]			
	1	2	3	4
Grasses				
Oryzopsis hymenoides	0.65	0.30	--	--
Agropyron smithii	16.20	--	--	--
unknown grasses	--	--	0.05	--
TOTAL	16.85[a]	0.30[a]	0.05[a]	0.00[a]
Forbs				
Astragalus spatulatus	0.05	--	0.05	--
Phlox hoodii	0.05	0.05	--	--
Salsola kali	1.00	55.60	--	1.00
unknown forbs	1.05	--	--	--
Descurania pinnata	4.60	--	--	--
Trifolium gymnocarpon	0.10	0.10	--	--
Chenopodium fremontii	3.20	--	--	--
C. album	--	0.40	--	--
Astragalus purshii	0.60	--	--	--
TOTAL	10.65[a]	56.15[a]	0.05[a]	1.00[a]
Shrubs				
TOTAL	0.00[a]	0.00[a]	0.00[a]	0.00[a]
Grasses + Forbs + Shrubs				
TOTAL	27.50[a]	56.45[a]	0.10[a]	1.00[a]

[1]See Table 3 for description of treatments.

[a]Means in the same row bearing different superscript letters are significantly different ($P<0.05$).

Table 9. Mean percentage cover of plant species and life forms per m^2 for each treatment in the 1977 Annual Disturbance Plot sampled in 1978.

Life Form/Species	Treatment[1]			
	1	2	3	4
<u>Grasses</u>				
Oryzopsis hymenoides	0.30	0.30	--	--
Agropyron smithii	3.40	--	--	--
unknown grasses	--	--	0.05	--
TOTAL	3.70[a]	0.30[a]	0.05[a]	0.00[a]
<u>Forbs</u>				
Astragalus spatulatus	0.05	--	0.05	--
Phlox hoodii	0.05	0.05	--	--
Salsola kali	0.80	8.00	--	0.60
unknown forbs	0.45	--	--	--
Descurania pinnata	2.80	--	--	--
Trifolium gymnocarpon	0.10	0.10	--	--
Chenopodium fremontii	0.60	--	--	--
C. album	--	0.20	--	--
Astragalus prushii	0.60	--	--	--
TOTAL	5.45[a]	8.35[a]	0.05[a]	0.60[a]
<u>Shrubs</u>				
TOTAL	0.00[a]	0.00[a]	0.00[a]	0.00[a]
<u>Grasses + Forbs + Shrubs</u>				
TOTAL	9.15[a]	8.65[a]	0.10[a]	0.60[a]

[1]See Table 3 for description of treatments.

[a]Means in the same row bearing different superscript letters are significantly different ($P<0.05$).

However, the weedy invader (Russian thistle, *Salsola kali*) accounted for the majority of this biomass. Grasses, forbs, and shrubs together exhibited density, biomass and cover trends similar to those for forbs. As can be seen in Tables 5-7, shrubs were unable to invade any of the 1977 treatments.

Ranked by treatment, grasses and forbs equally had the highest density, biomass, and cover on Treatment 1. None of the life forms were well established on Treatments 2-4. Forbs had the largest biomass and cover on Treatment 2 while essentially no plant biomass and cover were demonstrated on Treatments 3 and 4.

Plant species which were present on the 1977 plots but not present within the quadrats were as follows: Douglas dustymaiden (*Chaenactis douglasii*), penstemon, lobeleaf groundsel (*Senecio multilobatus*), nuttall goldenweed (*Haplopappus nutallii*), meadow milkvetch, knowtwed (*Polygonum* spp.), foothill bladderpod (*Lesquerella ludoviciana*), smallflower aster (*Aster arenosus*), fireweed summercypress (*Kochia scoparia*), bottlebrush squirreltail, streambank wheatgrass (*Agropyron riparium*), Douglas rabbitbrush, rubber rabbitbrush (*Chrysothamnus nauseosus*), big basin sagebrush (*Artemisia tridentata*), and pricklypear (*Opuntia polyacantha*).

Summary

Following the first year of disturbance the mean density of grasses, forbs, and shrubs was significantly highest on Treatment 1. This trend was attributed to high mean density of grasses. The primary grass species that established were prairie junegrass, Indian ricegrass, and wheatgrass. Part of the reason for the successful invasion of grasses may be attributed to the rhizomes of the wheatgrass being left behind which provided a reproductive base not similarly available to the forbs and shrubs. Forb density, biomass, and cover was high on Treatments 1 and 2 while shrubs failed to invade on any of the treatments. Negligible plant density, biomass, and cover was observed on Treatments 3 and 4 (the most severe treatments).

The second year following plot disturbance total plant density, biomass, and cover remained highest on Treatment 1. Grasses and forbs increased in density on all treatments with forbs making the greatest increase. Grasses, however, maintained the highest mean density on Treatment 1. Shrubs remained negligible on all treatments.

Overall, total plant density decreased with an increase in disturbance while total biomass and cover decreased in order of treatments--1, 3, 2, and 4. Forbs were responsible for the increase in biomass and cover on Treatment 3.

Retorted Shale Successional Study

During the summer and fall of 1977, a successional study on retorted shale was established at the Intensive Study Site. This study was implemented to address some of the problems the oil shale industry will face in the formation of a control technology for the surface disposal and revegetation of retorted oil shale. The experimental design of this study is based on the most recent surface disposal plans proposed by industry. Specifically, the study was set up to evaluate the effect of retorted shale properties and the method of disposal on the rate and direction of plant community succession. Various disposal schemes, species mixtures, and fertiizer treatments will be tested for their ability to aid in the reestablishment of diverse and functional ecosystems on retorted shale disposal sites.

Field Season 1977

Construction of the Retorted Shale Successional Study began in June 1977 with the removal of vegetation from six rectangular panels measuring 23x109 m. Five of the panels were then excavated to specified depths according to the designated profile configuration for each panel (Figure 25). Excavated topsoil and subsoil were stockpiled separately. Following excavation the long sides of each panel were lined with cresote treated plywood to confine root growth to the artificial soil-shale profile.

Paraho retorted shale was then transported from the Anvil Points facility west of Rifle, Colorado, to the Intensive Study Site. Each of the five excavated panels received 61 cm of retorted shale material. In order to simulate industry's proposals, the lower 15 cm of the retorted shale was compacted to a density range of 1,360 to 1,520 kg/cu m. The compaction was undertaken in an attempt to make the lower layer of retorted shale impervious to water movement and root penetration. The upper 46 cm of shale material in the profile was compacted to a density range of 1,200 to 1,360 kg/cu m by normal equipment traffic.

Topsoil, subsoil, and gravel material were then deposited over the retorted shale according to the experimental design (Figure 25). The gravel treatment in Panel 6 was composed of a coarse gravel layer of large pond rock (5-15 cm diameter) topped with a layer of fine gravel (less than 4 cm diameter). This layer will test the effectiveness of gravel as a barrier to the capillary rise of salts from the retorted

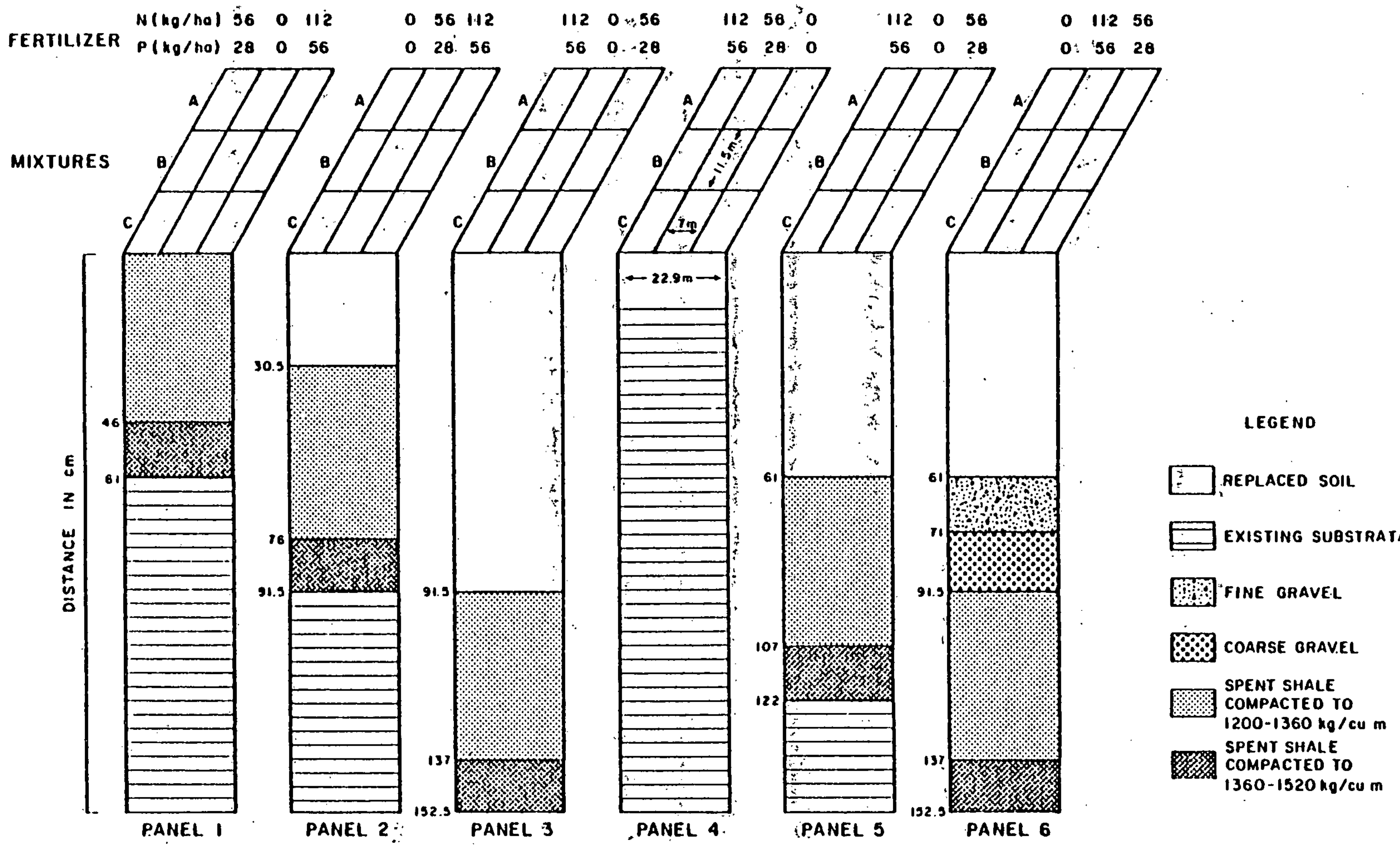

Figure 25. Profile configuration for Retorted Shale Successional Study using Paraho retorted shale.

shale to the overlying soil material. The control or soil check panel was ripped to a depth of 30 cm and graded to surface contour.

The completed, artificial soil-shale profiles are:

1. Processed shale without any surface covering,
2. 30.5 cm soil over retorted shale,
3. 91.5 cm soil over retorted shale,
4. Soil check with no retorted shale,
5. 61 cm soil over retorted shale, and
6. 61 cm soil over 30.5 cm capillary barrier over retorted shale.

The experimental design for the Retorted Shale Successional Study consists of three treatment variables (soil-shale profile, seed mixture, and fertilizer treatment) applied in a split-split block design (Figure 26). The basic design was replicated three times. Both the three seed mixtures and the three fertilizer treatments were randomized prior to application. The study was drilled with the three seed mixtures (Table 10) in the fall of 1977. The three fertilizer treatments were as follows:

Nitrogen	Phosphorus
112 kg/ha	56 kg/ha
56 kg/ha	28 kg/ha
0 kg/ha	0 kg/ha

Phosphorus was applied prior to seeding while the nitrogen was not applied until the end of the first growing season.

Replicate	Panel 1			Panel 2			Panel 3			Panel 4			Panel 5			Panel 6		
REPLICATE I	A1	A3	A2	B2	B1	B3	B2	B1	B3	A2	A3	A1	B1	B3	B2	B3	B2	B1
	C1	C2	C3	A2	A3	A1	C1	C3	C2	C3	C1	C2	C3	C1	C2	A1	A3	A2
	B3	B2	B1	C1	C2	C3	A2	A3	A1	B1	B2	B3	A2	A1	A3	C2	C3	C1
REPLICATE II	A3	A1	A2	B1	B3	B2	B3	B1	B2	B2	B3	B1	A1	A3	A2	B2	B1	B3
	B2	B3	B1	C1	C2	C3	A2	A3	A1	C2	C1	C3	B3	B2	B1	C2	C3	C1
	C1	C3	C2	A1	A2	A3	C2	C1	C3	A3	A2	A1	C2	C3	C1	A2	A3	A1
REPLICATE III	A2	A3	A1	A3	A1	A2	A3	A1	A2	B2	B1	B3	C2	C3	C1	A1	A3	A2
	C1	C3	C2	C1	C2	C3	C1	C3	C2	C3	C2	C1	B1	B3	B2	C3	C1	C2
	B1	B2	B3	B2	B1	B3	B2	B3	B1	A1	A2	A3	A3	A2	A1	B1	B3	B2

Mixture

A = Combination native and introduced mixture
B = Native mixture
C = Introduced mixture

Fertilization Rate

1 = 112 kg/ha N, 56 kg/ha P
2 = 56 kg/ha N, 28 kg/ha P
3 = 0 kg/ha N, 0 kg/ha P

Figure 26. Experimental design for Retorted Shale Successional Plot.

Table 10. Seeding rates for three species mixtures used on Retorted Shale Successional Plots.

	Common Name	Scientific Name	Seeding Rate PLS (kg/ha)
Mixture A--Combination, native and introduced species			
1.	Nordan crested wheatgrass	*Agropyron cristatum*	1.12
2.	Siberian wheatgrass	*A. sibiricum*	1.12
3.	Critana thickspike wheatgrass	*A. dasystachyum*	1.12
4.	Sodar streambank wheatgrass	*A. riparium*	1.12
5.	Slender wheatgrass	*A. trachycaulum*	1.12
6.	Regar meadow brome	*Bromus erectus*	1.12
7.	Indian ricegrass	*Oryzopsis hymenoides*	1.12
8.	Green needlegrass	*Stipa viridula*	1.12
9.	Durar hard fescue	*Festuca ovina duriuscula*	.56
10.	Madrid yellow sweetclover	*Melilotus officinalis*	.56
11.	Utah sweetvetch	*Hedysarum boreale utahensis*	1.12
12.	Globemallow	*Sphaeralcea munroana*	.56
13.	Lewis flax	*Linum lewisii*	.56
14.	Arrowleaf balsamroot	*Balsamorhiza sagittata*	1.12
15.	Fourwing saltbush	*Atriplex canescens*	1.12
16.	Stansbury cliffrose	*Cowania mexicana stansburiana*	1.12
17.	Winterfat	*Ceratoides lanata*	1.12
18.	Green ephedra	*Ephedra viridis*	1.12
Mixture B--Native species			
1.	Rosana western wheatgrass	*Agropyron smithii*	1.12
2.	Sodar streambank wheatgrass	*A. riparium*	1.12
3.	Bearded bluebunch wheatgrass	*A. spicatum*	1.12
4.	Indian ricegrass	*Oryzopsis hymenoides*	1.12
5.	Green needlegrass	*Stipa viridula*	1.12
6.	Durar hard fescue	*Festuca ovina duriuscula*	.56
7.	Shermans big bluegrass	*Poa ampla*	1.12
8.	Alkali sacaton	*Sporobolus airoides*	.56
9.	Globemallow	*Sphaeralcea munroana*	.56
10.	Utah sweetvetch	*Hedysarum boreale utahensis*	1.12
11.	Palmer penstemon	*Penstemon palmeri*	.56
12.	Stansbury cliffrose	*Cowania mexicana stansburiana*	2.24
13.	Green ephedra	*Ephedra viridis*	1.12
14.	Fourwing saltbush	*Atriplex canescens*	1.12
15.	Winterfat	*Ceratoides lanata*	1.12
16.	Antelope bitterbrush	*Purshia tridentata*	1.12
Mixture C--Introduced species			
1.	Nordan crested wheatgrass	*Agropyron cristatum*	1.12
2.	Siberian wheatgrass	*A. sibiricum*	1.12
3.	Jose tall wheatgrass	*A. elongatum*	1.12
4.	Luna pubescent wheatgrass	*A. trichophorum*	1.12

Table 10.--Continued

	Common Name	Scientific Name	Seeding Rate PLS (kg/ha)
5.	Oahe intermediate wheatgrass	*A. intermedium*	1.12
6.	Manchar smooth brome	*Bromus inermis*	1.12
7.	Regar meadow brome	*B. erectus*	1.12
8.	Vinal Russian wildrye	*Elymus junceus*	1.12
9.	Ladak alfalfa	*Medicago sativa*	.56
10.	Madrid yellow sweetclover	*Melilotus officinalis*	.56
11.	Lutana cicer milkvetch	*Astragalus cicer*	.56
12.	Sainfoin	*Onobrychis viciaefolia*	.56
13.	Bouncing bet	*Saponaria officinalis*	1.12
14.	Small burnet	*Sanguisorba minor*	2.24
15.	Siberian peashrub	*Caragana arborescens*	1.12
16.	Russian olive	*Elaeagnus angustifolia*	2.24

Field Season 1978

In order to adequately monitor plant establishment and subsequent successional trends, a comprehensive sampling program was initiated on the Retorted Shale Successional Study in 1978. Six randomly located, permanently placed, rectangular-shaped 0.25 m^2 quadrats were established in each subplot. The quadrat location was marked by the placement of steel stakes in opposite corners. Primary vegetative parameters measured were density, cover, and biomass by individual plant species. Biomass measurements were obtained by correlating nonconsumptive estimates from the subplots with estimates and clipped plants taken from the buffer zones. Plant vigor was monitored through the measurement of maximum plant height and seed stalk production. Both maximum plant height and number of seed stalks were taken on a per species per quadrat basis.

The Retorted Shale Successional Study was sampled twice during the 1978 growing season. The initial sampling in mid June provided early establishment data, while the sampling in late August produced data on first-year survival. During the late season sampling period, it was felt that the permanent quadrats were not giving us an adequate estimate of plant invasion on the subplots. A sampling procedure was then undertaken utilizing the whole subplot as a quadrat to more fully document plant invasion. The subplots were walked in an east-west direction in 1 m strips by two researchers who recorded basal area and biomass of any invading species. These data are currently being reduced and analyzed to give a more complete picture of plant invasion on each treatment.

In addition to vegetation sampling, soil and shale samples were taken from the artificial profiles during the field season. Surface soil samples were collected in August in an attempt to explain biomass differences measured between the various soil-shale profiels. Composite samples were taken from each replicate of the five panels that contain surface soil and sent to the Colorado State University Soil Test Laboratory for analysis of total nitrogen, nitrate, and ammonium concentration. The results (Appendix B, Table 1) showed that the biomass differences between soil-shale profiles could not adequately be explained by differences in nitrogen availability to the plants.

Soil and shale samples were taken at various depths from the artificial profiles in November 1978 (Appendix B, Table 2). This sampling is a part of our monitoring program to determine chemical and physical changes that occur in the soil-shale profiles through time.

Subsurface soil moisture readings in the artificial soil-shale profiles were taken biweekly throughout the growing season using a nuclear probe. Readings were taken at each of the study's 18 stations (one station per replicate per panel) at depths of 15, 30, and 45 cm. The results of this work show several interesting trends (Appendix B, Figures 1 and 2).

Higher subsurface moisture readings were maintained in the retorted shale material than in the soil material as the growing season progressed. At the end of the growing season moisture readings in the shale (all readings in Panel 1 and the 30 cm and 45 cm readings in Panel 2) were much higher than in the soil material of the other profiles. Moisture readings in the soil material were lower because the water was being used by the actively growing plants. Since little vegetation growth took place in the shale the water was not used and, therefore, accumulated.

The soil check (Panel 4) exhibited consistantly lower moisture readings at all depths through the growing season. This may be a result of lower infiltration rates which reduce storage and tend to keep the moisture in the upper layer of the profile where it is more subject to evaporation.

Consistently high moisture readings were obtained at the 30 and 45 cm depths in Panel 6 (61 cm soil over 30.5 cm gravel). This increased moisture may be accounted for by the presence of the gravel layer acting as a capillary barrier. Water in the profile is unable to move across the gravel layer and is stored in the overlying soil material.

Relatively high moisture readings were also observed in Panel 3 (91.5 cm soil cover). The large amount of soil material in the profile may have allowed for greater moisture storage which resulted in higher readings.

Surface soil moisture readings were also taken biweekly through the use of a calcium carbide, gas pressure, moisture tester. Data taken from the 18 stations (one station per replicate per panel) shows that the surface moisture response was similar to that of subsurface moisture (Appendix B, Figure 3).

Panel 6 had the highest surface soil moisture readings while Panel 4 had the lowest. The high readings on Panel 6 for surface soil moisture are most likely caused by the presence of the capillary gravel barrier in the profile. The relatively high surface soil moisture readings on Panel 2 can be explained in part by the change in texture between the soil and underlying shale material. This textural change between the two materials would tend to restrict water flow and keep more moisture in the upper profile. Panels 3 and 5 had surface soil moisture readings intermediate between the high of Panel 6 and the low of Panel 4.

Soil temperature-moisture cells were installed on each sub-subplot in the northern and southern replicate of each panel. They were buried at a depth of 15 and 45 cm and will be monitored during the upcoming field season.

Plywood paneling was installed on the northern and southern ends of each panel during the 1978 field season. With this installation, each panel is now completely encompassed by plywood paneling to the lower depth of the profile configuration. This was done to insure that root growth is confined to the artificial soil-shale profile and does not grow into adjacent buffer areas.

A rodent-proof fence was constructed around the Retorted Shale Successional Study this past year. It will minimize plant damage from small herbivores and enable us to adequately assess plant response to the various treatments.

Results

The data obtained during the first sampling year was reduced and then analyzed statistically. An analysis of variance was run on all subplot treatment means for biomass, cover, and density by life form. Tukey's Q-tests were then utilized to determine significant differences between treatment means.

All the results presented in the following section are based on data collected during the August 1978 sampling. The response from the shale-to-surface treatment (Panel 1) is nearly always significantly different from the response on the other five panels. This is because a stand of vegetation failed to become established on the shale-to-surface panel. Analysis of the data on a species basis for all vegetative parameters is currently being done.

Effect of Seed Mixtures on the Density of Grasses, Forbs, and Shrubs

The total density of seeded and invading plant species showed a varying response to the different seed mixtures. The introduced seed mixture responded with a significantly higher (P=.0325) density of plants than the native seed mixture. Total density of the introduced seed mixture was also greater than the combination seed mixture, but this difference was not significant at the .05 level. This greater density response from the introduced seed mixture may be the result of two factors. One, the introduced plant species are generally more aggressive and establish themselves better than native plant species. Two, the introduced seed mixture allowed a greater number of invading species to become established.

Total Density of Invading Species. Examination of the total density of invading species showed a significant interaction (P=.0265) between seed mixture and soil-shale profile (Figure 27). Panels 2 and 4 appeared to have a greater density of invading species. The greater density of invasion on Panel 4 may be the result of an indigenous seed source which was not present on the other panels. The greater density of invading species on Panel 2 may be, in part, explained by the lower amount of total biomass of seeded species observed on this panel. It is believed that the lower biomass of seeded species on Panel 2 left more open spaces available to the opportunistic invading plant species. It should be

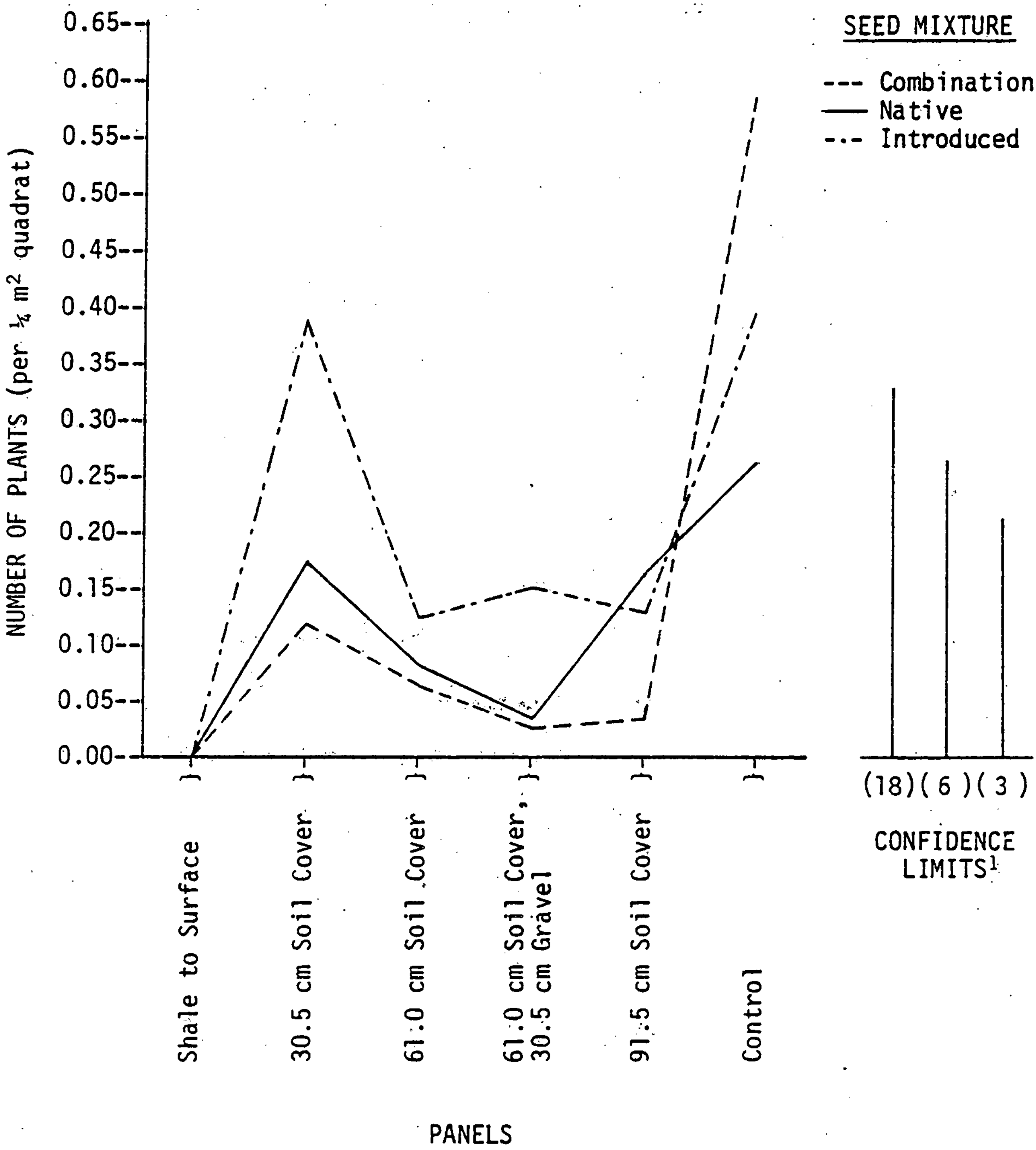

Figure 27. Total density of grasses, forbs, and shrubs of invading species on the Retorted Shale Successional Plots.

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[1]The honest significant difference procedure or Tukey's Q-test was used to determine significant differences between means in a significant main effect or interaction. Confidence intervals are shown above the number of means to be compared. Within interactions a group of means representing all levels of one treatment and at one level of the other treatments can be compared using the confidence interval above the number of means in the group.

noted here that those panels which had the higher levels of biomass production also contained the smaller number of invading species.

The total density of invading species showed differeing response according to seed mixture. On the five excavated panels (this excludes Panel 4, soil check) the introduced seed mixture generally allowed the greatest number of invading species followed by the native and then the combination seed mixture. The seed mixture composed of introduced species exhibited the lowest biomass of seeded species across all six panels and all three seed mixtures. The combination of native and introduced species, which generally had the highest biomass of seeded species, allowed the lowest density of invading species. A seed mixture that is showing high biomass production is making more complete use of resources (light, water, and nutrients) available at that particular site. When this occurs, less resources or open space is available for the invading plant species to utilize, and lower numbers of invading species result.

Total Density of Forbs. The total density of forb species, both seeded and invading, showed a significant interaction (P=.0004) between seed mixture and soil-shale profile. In most cases, a greater density of forbs was observed in the introduced seed mixture than either the combination or native seed mixtures (Figure 28). These differences can be partially explained by the number of forbs seeded and the seeding rate of forbs per seed mixture. They were:

Seed Mixture	Number of Forbs	Seeding Rate PLS (kg/ha)
1--Combination	5	3.82
2--Native	3	2.24
3--Introduced	6	5.60

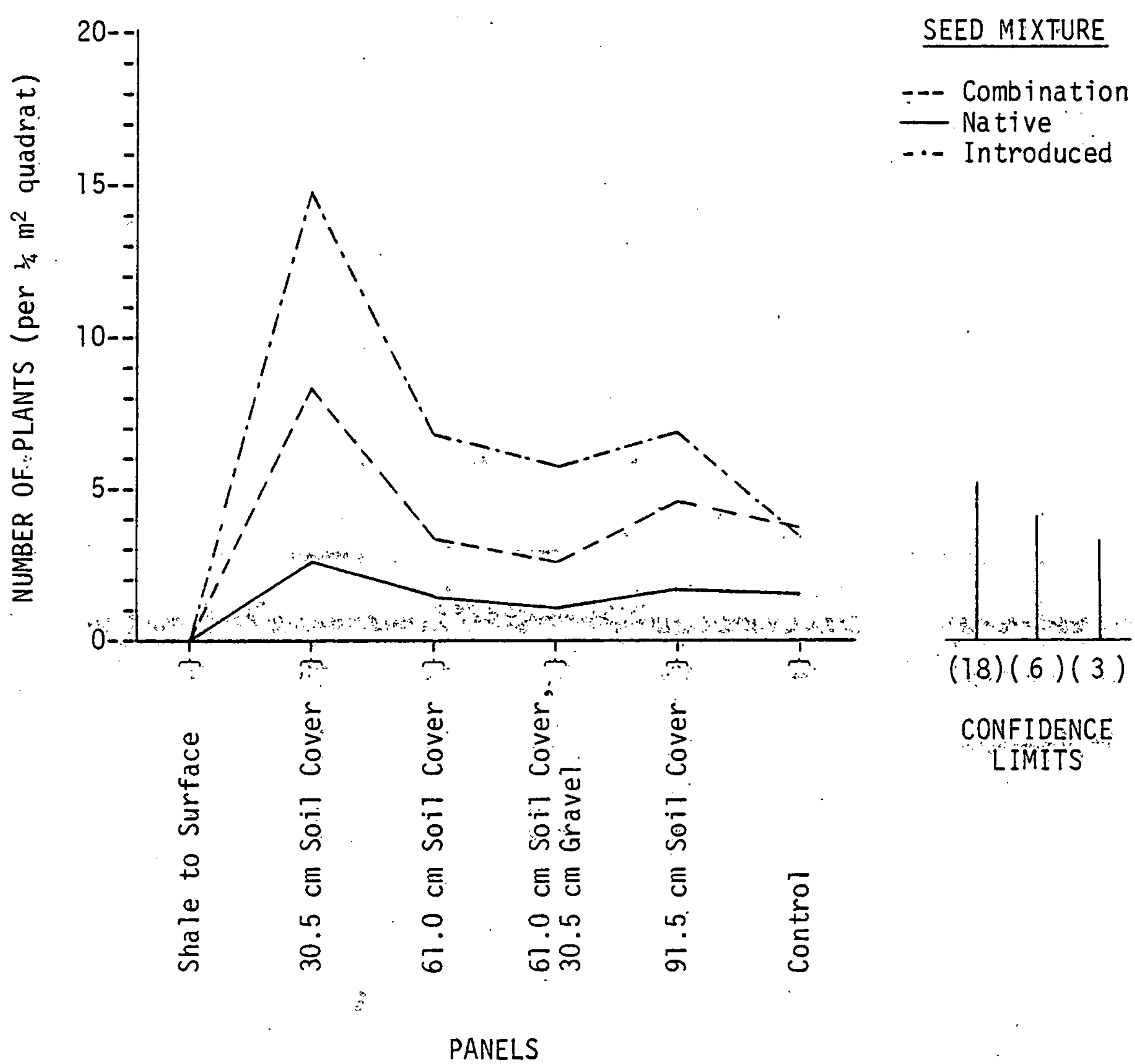

Figure 28. Total density of forbs for seeded and invading species on the Retorted Shale Successional Plots.

Thus, the density response of forbs by seed mixture can be directly correlated with the number of seeding rate of forb species per seed mixture.

Total density of seeded and invading forb species was greatest on Panel 2 than any of the other five panels. The reason Panel 2 is showing a higher density of forb species is not fully understood at this time.

The total density of seeded forbs also shows a significant interaction between seed mixture and panel (Appendix B, Table 9). This interaction is much the same as that discussed above for total density of seeded and invading forbs (Figure 29).

The interaction between seed mixture and panel was also significant (P=.0011) when looking at the density of invading forb species (Figure 30). Invading forb density is greatest on Panels 2 and 4. The higher invading forb density on Panel 4 may be a result of the indigenous seed source. The introduced seed mixture allowed the greatest density of invading forbs on all panels except Panel 4.

Total Density of Shrubs. The total density of shrubs, both seeded and invading species, exhibited a significant interaction between seed mixture and panel (Figure 31). The native seed mixture had a consistantly higher shrub density than either the combination or introduced seed mixtures. In Panel 2 both the native and combination seed mixtures were significantly greater in shrub density than the introduced seed mixture. The shrub density of the native seed mixture was significantly greater than that of the introduced seed mixture on Panels 3, 5, and 6. The extremently poor density response of shrubs in the introduced seed mixture can in part be explained by two factors. First, the number of

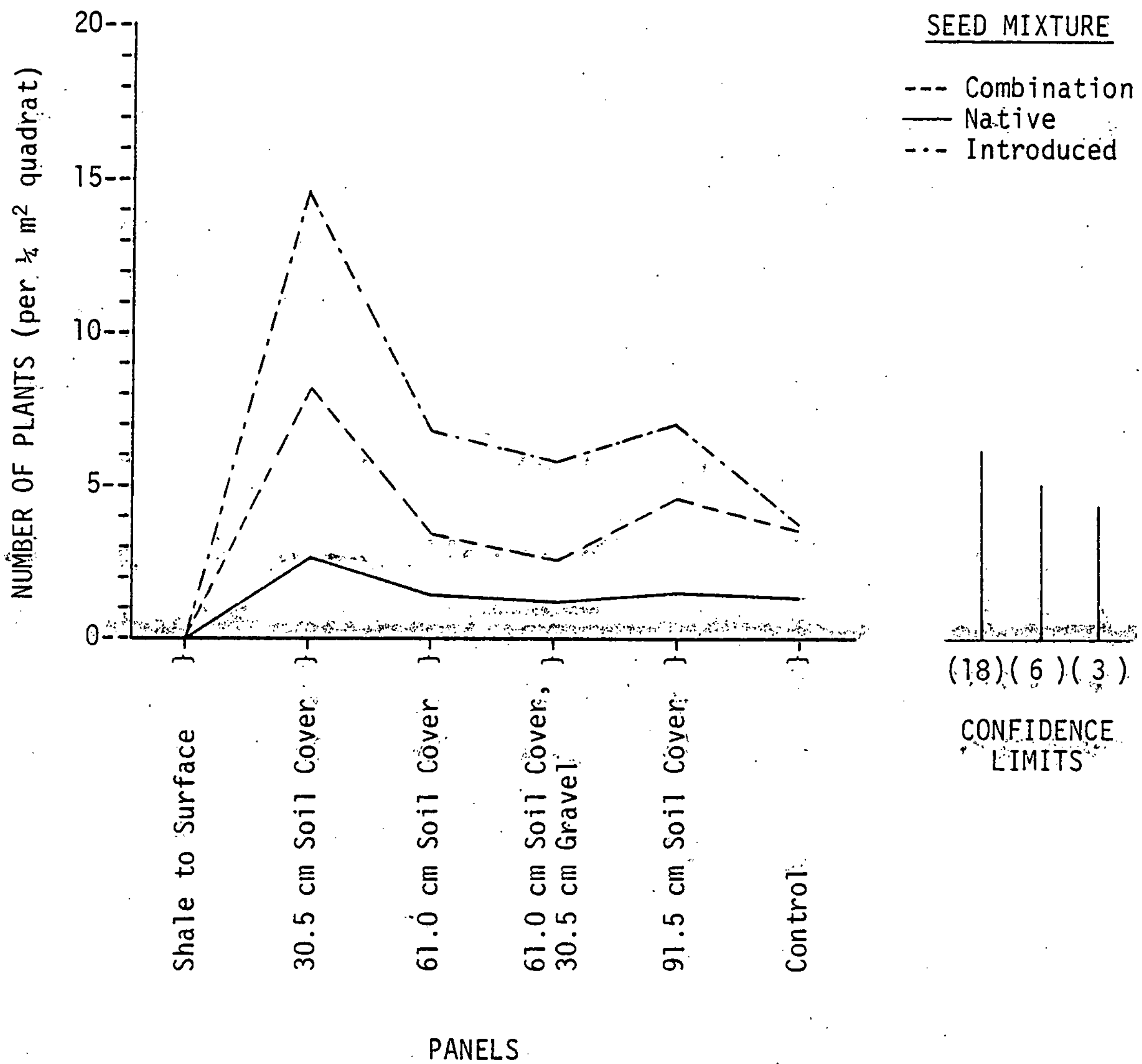

Figure 29. Total density of seeded forbs on the Retorted Shale Successional Plots.

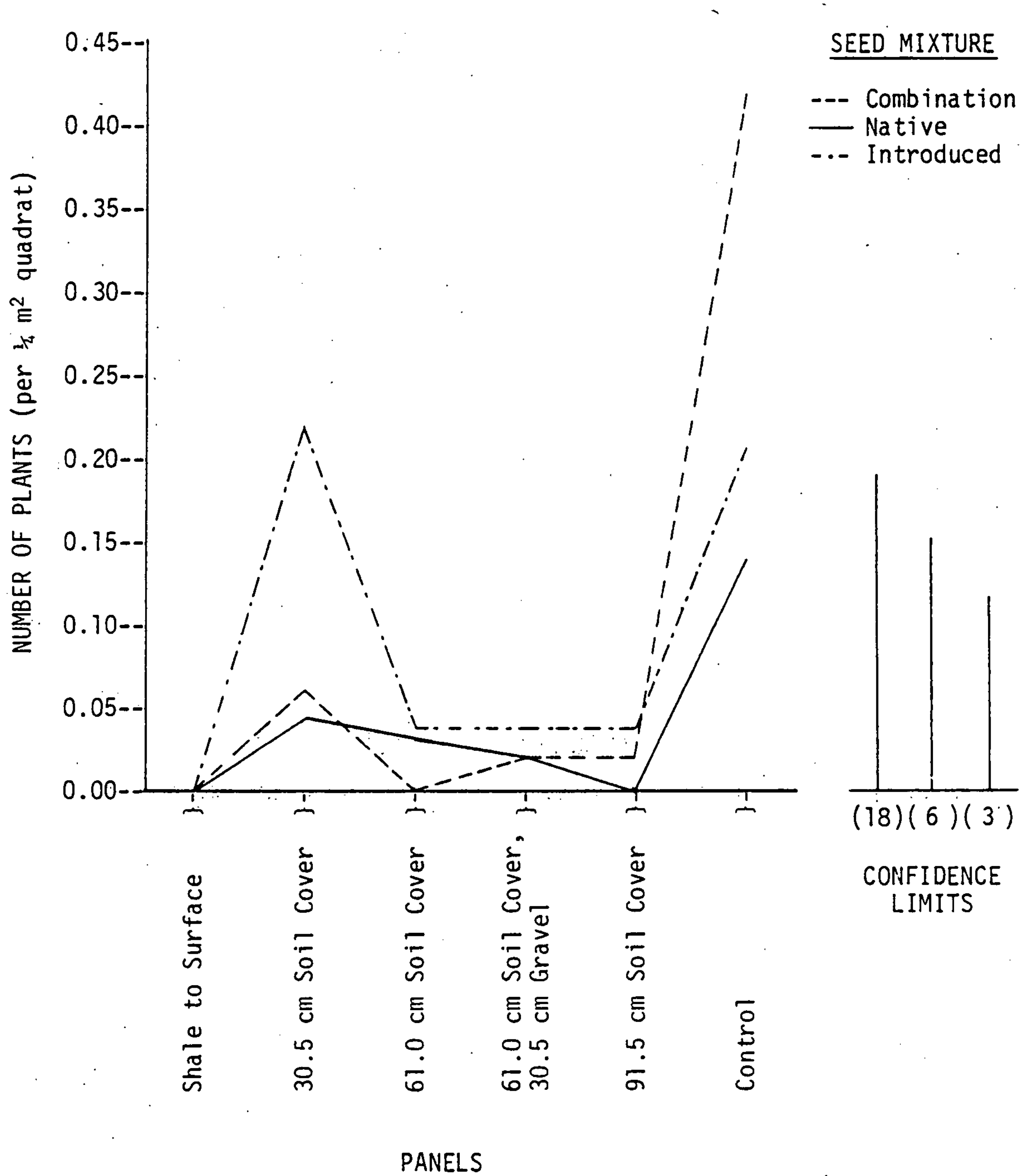

Figure 30. Total density of invading forbs on the Retorted Shale Successional Plots.

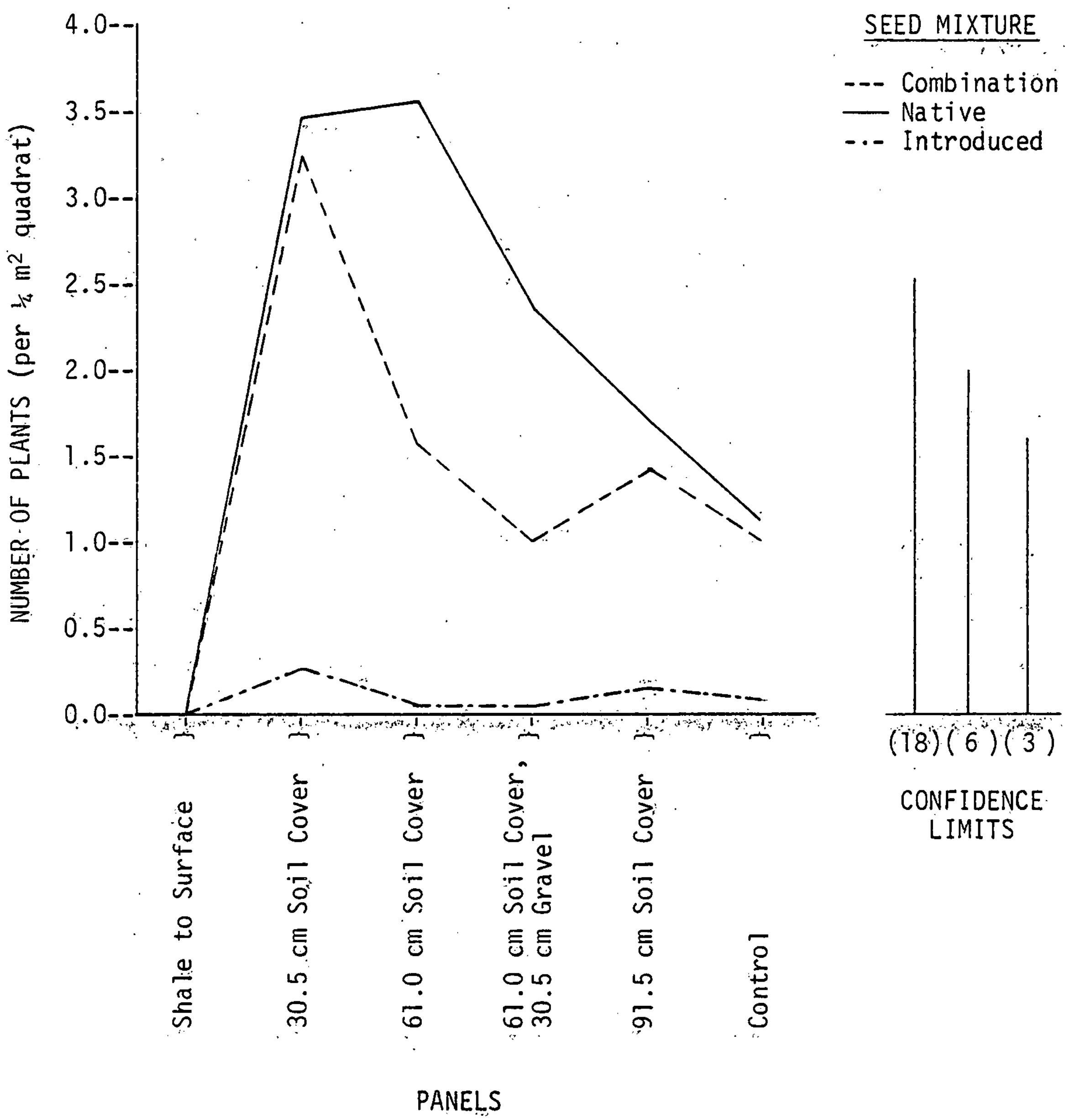

Figure 31. Total density of shrubs for seeded and invading species on the Retorted Shale Successional Plots

shrubs and the seeding rate of shrubs per seed mixture were not equivalent. They were:

Seed Mixture	Number of Shrubs	Seeding Rate PLS (kg/ha)
1--Combination	4	4.48
2--Native	5	6.72
3--Introduced	2	3.36

Thus, shrub density response can be correlated directly with the number and seeding rate of shrub species per seed mixture. In addition, the two shrub species in the introduced seed mixture performed poorly. Russian olive (*Elaeagnus angustifolia*) showed poor growth response while the Siberian peashrub (*Caragana arborescens*) was almost a total failure.

Shrub density of seeded species followed a nearly identical pattern to that of the total seeded and invading species (Figure 32). This was because invasion of shrub species on the study were minimal. Of the small amount of shrub invasion that occurred, a significantly greater number of invading shrubs were found in the introduced seed mixture than in either the combination or native seed mixtures.

Effect of Seed Mixture on the Biomass of Grasses, Forbs, and Shrubs

An interaction between seed mixture and fertilization was detected at the .0779 level for the total biomass of seeded and invading species (Appendix A, Table 10). The combination seed mixture showed a much better response to Fertilization Treatments 1 and 2 (varying levels of phosphorus only) than did either the native or introduced seed mixtures (Figure 32). But, when fertilizer was not applied, the native seed mixture had significantly higher biomass than the introduced seed mixture.

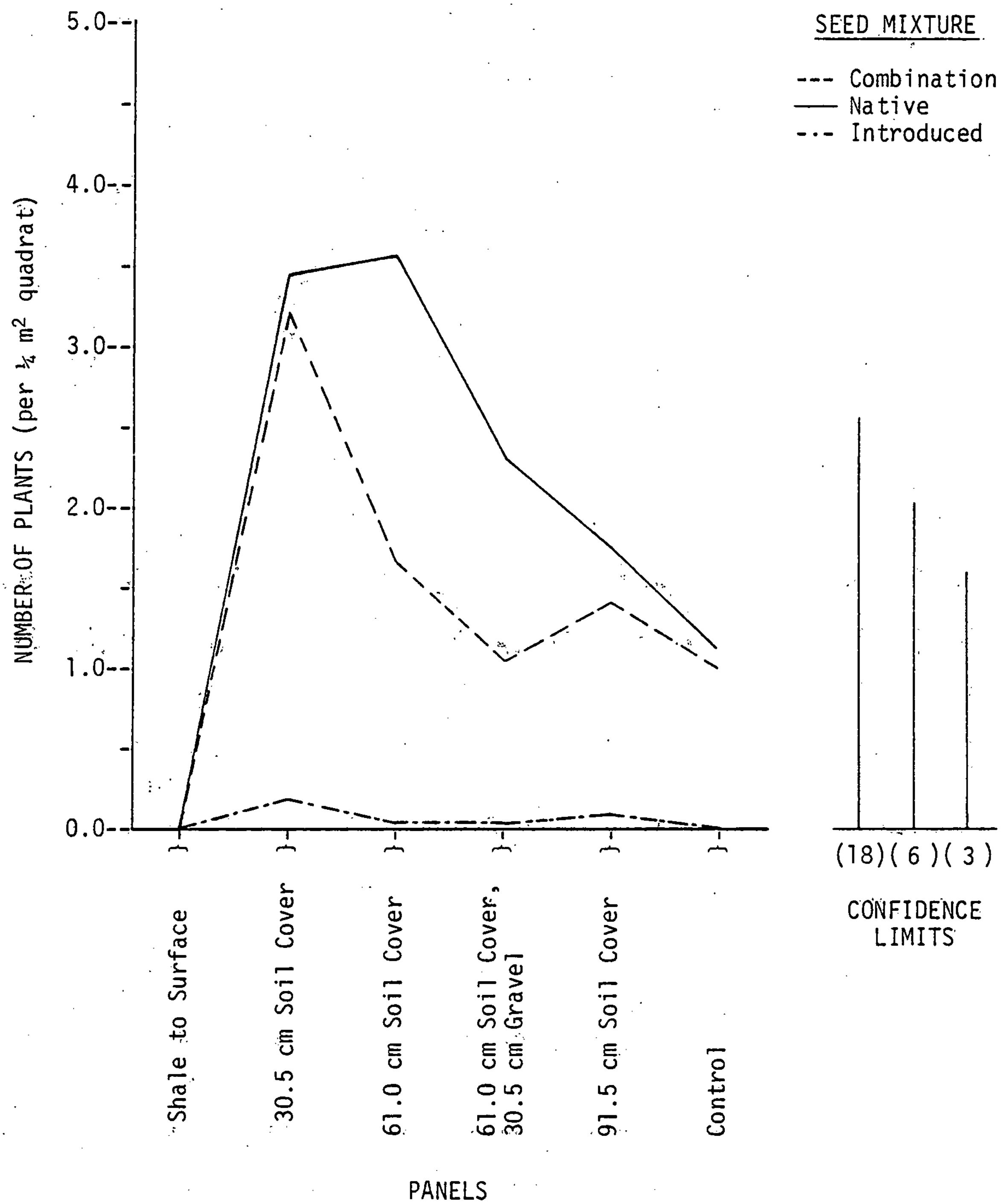

Figure 32. Total density of seeded shrubs on the Retorted Shale Successional Plots.

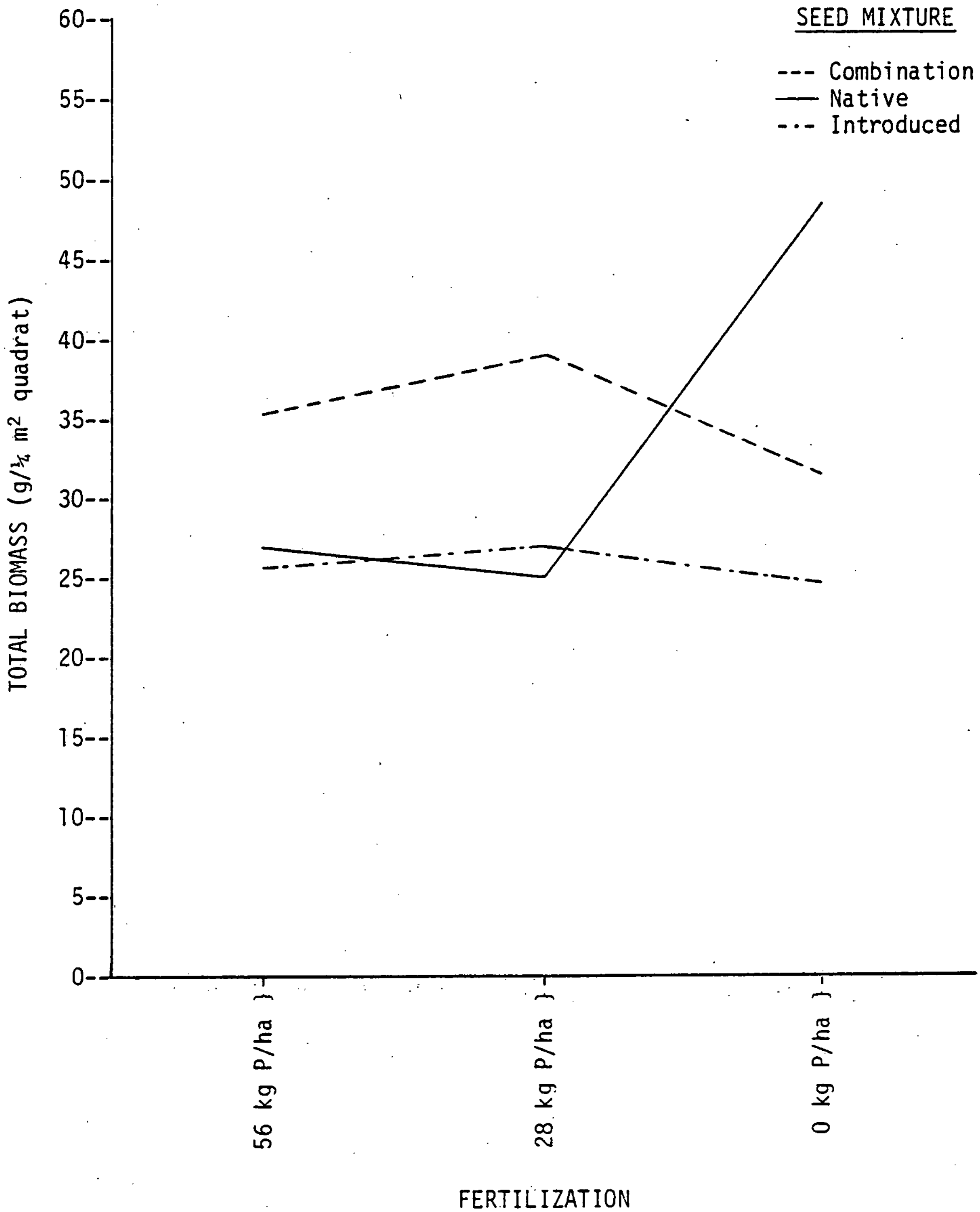

Figure 33. Total biomass of seeded and invading species on the Retorted Shale Successional Plots.

This can be explained in part by the greater adaptability of native plant species to the lower nutrient levels found in native rangelands.

Total Biomass of Seeded Species. There is a trend across all three seed mixtures for higher biomass production on the deeper soil-shale profiles (Panels 3, 5, and 6)(Figure 34). This greater biomass production on the deeper profiles is caused in part by the ability to store an increased amount of soil moisture. This fact is supported by subsurface moisture readings within the profiles as reported earlier.

The combination seed mixture had the highest overall biomass of any seed mixture across all panels. It showed a dramatic rise in biomass production on the deepest soil-shale profiles (Panels 3 and 6). The native seed mixture was intermediate in biomass production while the introduced seed mixture had the lowest overall production. Although the introduced seed mixture had the lowest biomass overall, it had the greatest biomass production on Panel 4. This was somewhat unusual as competition was the greatest on Panel 4 because of the indigenous seed source present.

Total Biomass of Grasses. The total biomass of seeded and invading grass species varied significantly (P=.0155) according to seed mixture. Plots seeded with the introduced seed mixture had significantly higher biomass of seeded and invading grass species than those seeded with the native seed mixture. This same response was also observed when seeded grass species were examined separately. But, when looking at the biomass of only invading species, the combination seed mixture contained a significantly greater biomass than either the native or introduced seed mixtures. The introduced seed mixtures which had the greatest overall

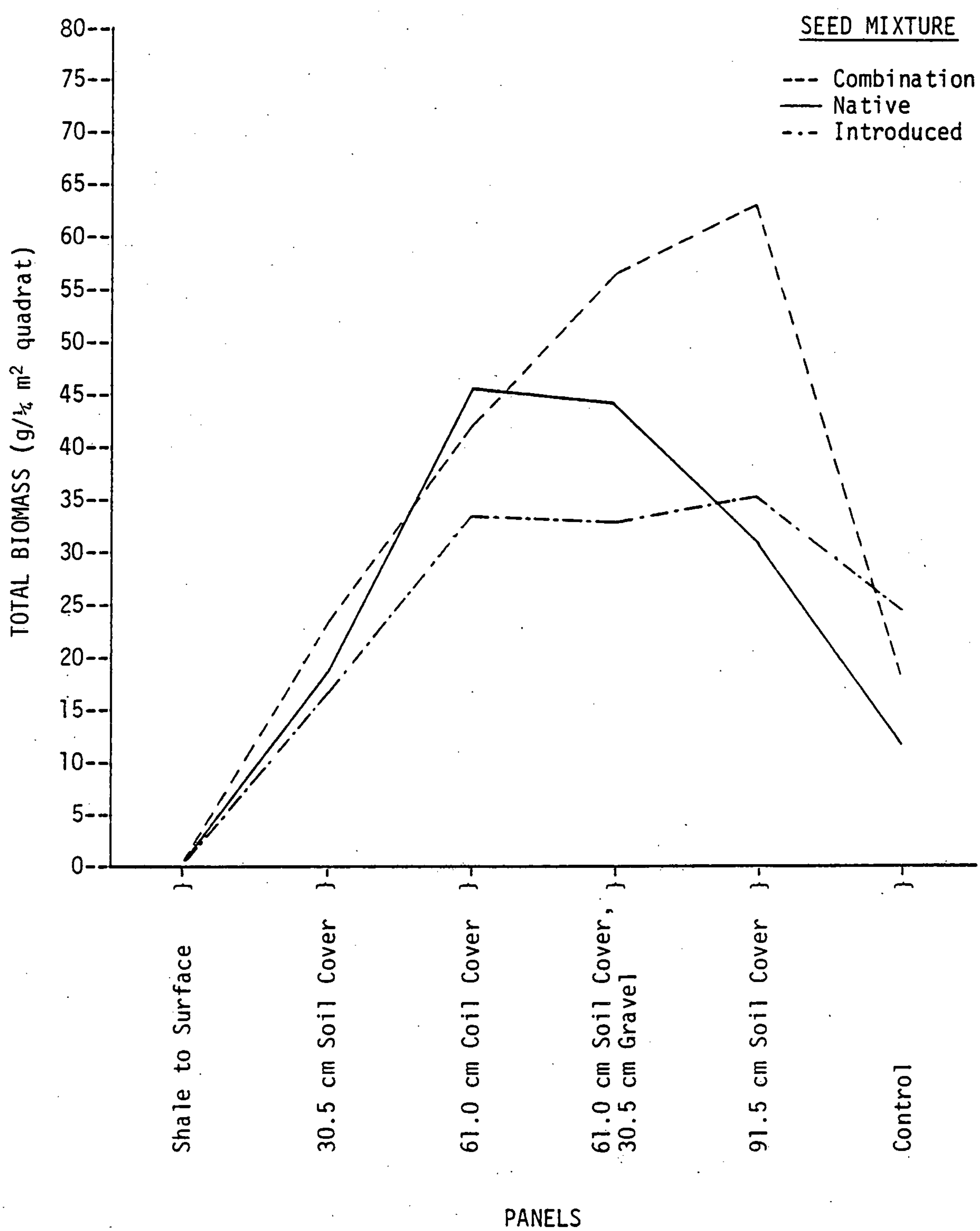

Figure 34. Total biomass of seeded species on the Retorted Shale Successional Plots.

biomass of grass species also allowed the lowest biomass of invading grass species.

The biomass of invading grass species showed a significant response (P=.0011) between seed mixture and panel (Figure 35). On Panel 3 the biomass of invading grass species was significantly higher in the native seed mixture than in the other two seed mixtures. Although the native seed mixtures generally showed a higher biomass of invading grass species across all panels, the reason for this sharp increase in Panel 3 is not known at this time.

Total Biomass of Forbs. The only significant difference observed in this section was among the seed mixtures as they influenced the total biomass of seeded forbs (Appendix A, Table 11). The combination seed mixture contained significantly higher biomass than the native seed mixture and higher biomass than the introduced seed mixture. These results of seeded forb biomass by seed mixture directly correlate with the number and seeding rate of forbs per seed mixture as presented earlier.

Total Biomass of Shrubs. Significant differences were seen in shrub biomass according to seed mixture when the total shrub biomass for seeded and invading species was examined. This same response was observed when seeded shrub species were examined separately. In both cases, the combination and native seed mixtures had significantly greater shrub biomass than the introduced seed mixture. The low shrub biomass in the introduced mixture is caused in part by the low seeding rate and the poor growth response of the introduced shrub species.

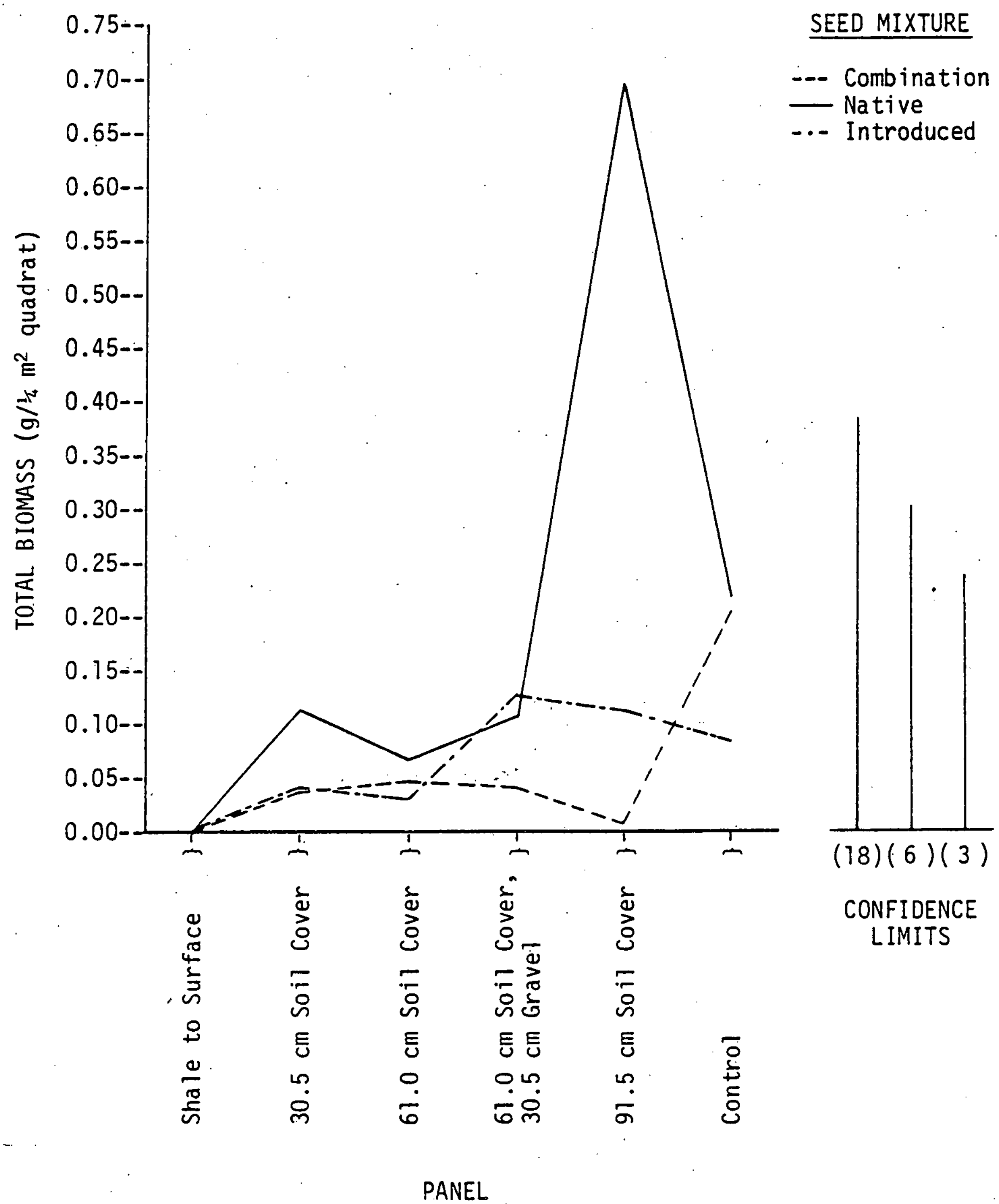

Figure 35. Total biomass of invading grasses on the Retorted Shale Successional Plots.

Effect of Seed Mixture on the Cover of Grasses, Forbs, and Shrubs

Variation in cover values is so great among the subplots that few significant differences or interactions were observed in response to the seed mixtures (Appendix A, Tables 11 and 12). Seed mixture caused a significant interaction or difference in only three areas in the cover of grasses, forbs, and shrubs.

Total Cover of Grasses. A significant interaction was observed between seed mixture and panel with respect to the cover of invading grass species (Figure 36). On Panel 3 the cover of invading grass species was significantly higher in the native seed mixture than in the other two seed mixtures. This sharp increase in cover values of invading grasses on Panel 3 with the native seed mixture is the same response noted earlier for biomass of invading grass species.

Total Cover of Shrubs. Total cover of shrubs, both seeded and invading species, showed significant (P=.0047) differences in cover by seed mixtures. Subplots seeded with combination or native seed mixtures had significantly higher shrub cover values than those seeded with the introduced seed mixture. The cover of seeded shrub species was greater in plots where the native seed mixture was seeded than where the introduced seed mixture was seeded (Appendix A, Table 12). These results correlate closely with those obtained for the biomass of shrub species.

Effect of Fertilization on the Density of Grasses, Forbs, and Shrubs

There are no significant interactions or differences when considering the density of grasses, forbs, and shrubs for the three fertilization treatments. The subplots showed too much variability in

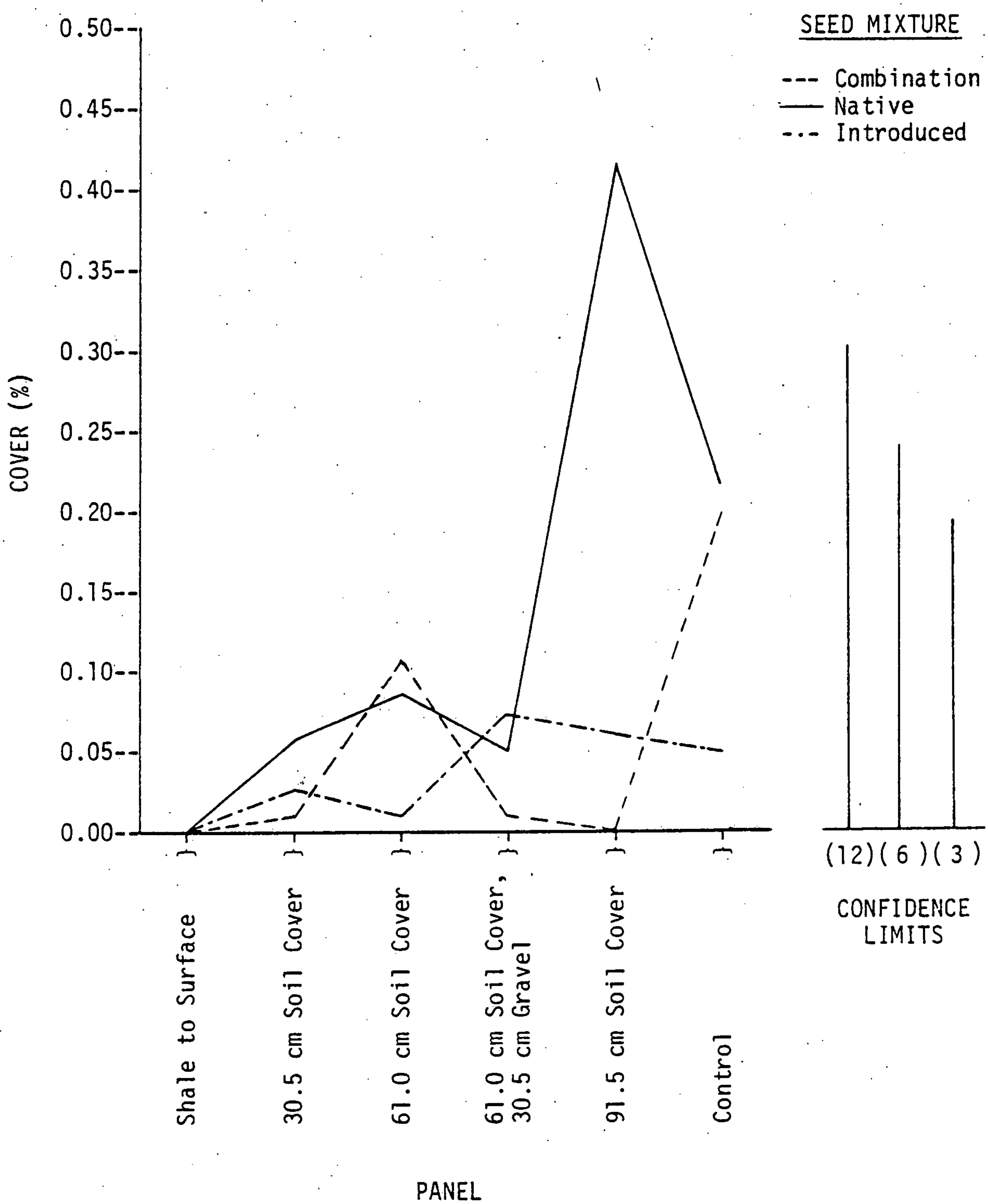

Figure 36. Total cover of invading grasses on the Retorted Shale Successional Plots.

density values to exhibit any significant effects caused by the different levels of fertilization.

Effect of Fertilization on the Biomass of Grasses, Forbs, and Shrubs

The only significant response of the biomass of grasses, forbs, and shrubs to fertilization occurred in the case of forbs in the seed mixtures. Seeded forbs showed a significant interaction between fertilization and panel with respect to biomass (Figure 37). On Panel 6 the plots fertilized with 28 kg P/ha had a significantly greater biomass of seeded forbs than those fertilized with 56 kg P/ha. Biomass response of seeded forbs was significantly higher within the nonfertilized treatment than within the highly fertilized treatment on Panel 3. The data suggests that higher fertilization levels of phosphorus may have a detrimental effect on seed forb biomass, especially in the deeper soil-shale profiles.

Effect of Fertilization on the Cover of Grasses, Forbs, and Shrubs

The cover of grasses, forbs, and shrubs showed no significant interactions or differences among fertilization treatments. The high amount of variability in cover values on the subplots caused the failure to see any significant cover response from the fertilization treatments.

Conclusions

1. Paraho retorted shale, without large inputs of resources and management, cannot be directly revegetated within an environmentally acceptable time period.
2. The data suggests that deeper soil coverings of 61 and 91.5 cm may be necessary over retorted shale to establish diverse and productive ecosystems.

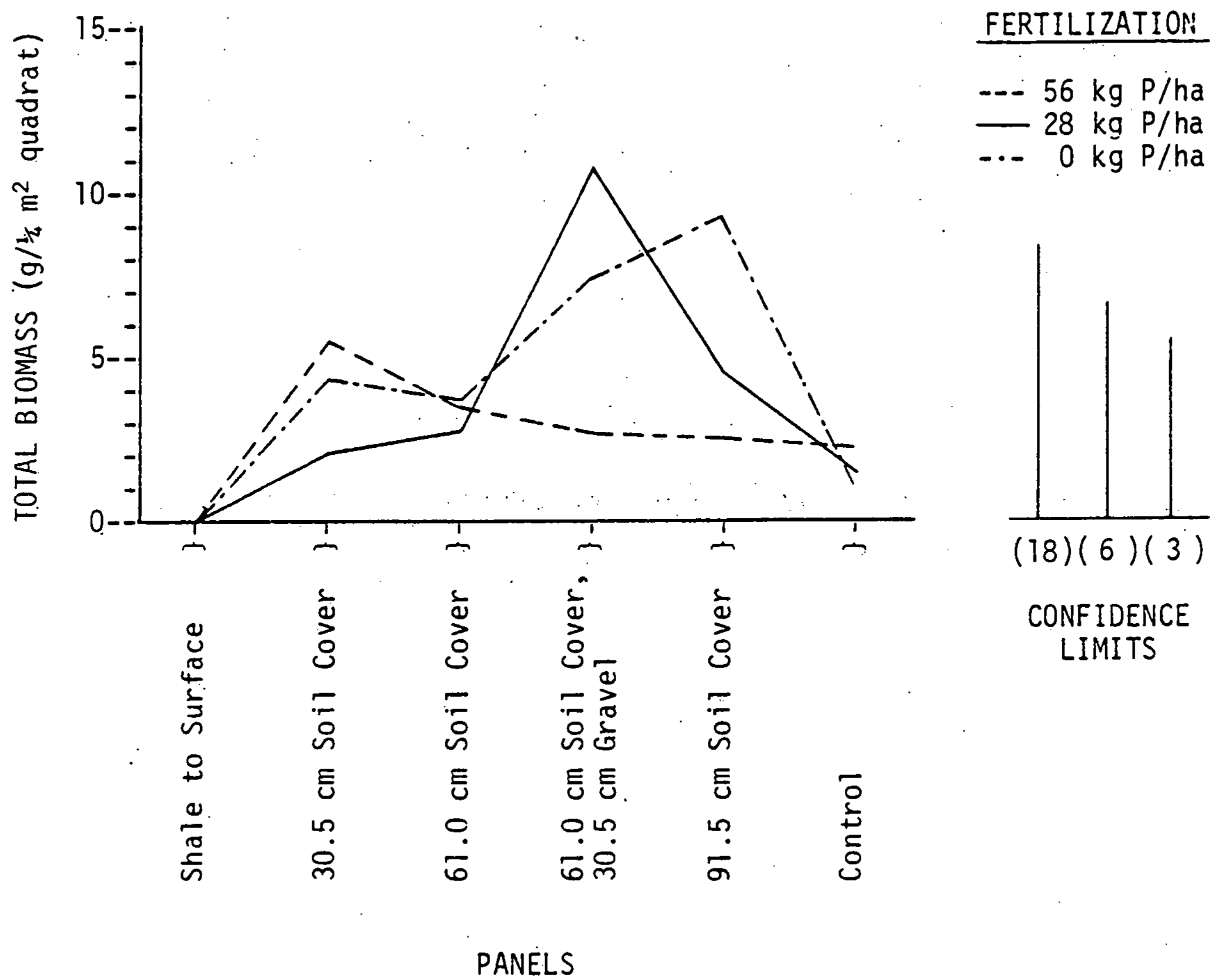

Figure 37. Total biomass of seeded forbs on the Retorted Shale Successional Plots.

3. Subplots seeded with the mixture composed of both introduced and native species responded with the highest biomass.
4. Subplots seeded with the introduced seed mixture allowed the greatest amount of invasion. This indicates that the introduced seed mixture was unable to form a closed community and thus was open to invasion.
5. Phosphorus fertilization had little effect on plant establishment.

Germination and Establishment Studies

Results

An extensive review of literature has been completed for Colorado native shrubs and submitted to the U.S. Forest Service for publication. This publication represents the first of three volumes addressing the areas of basic range seed physiology and the germination characteristics of native or wild plants. Volumes 2 and 3 are still in progress and will be finalized in the fall of 1979. Each volume will be useful for the development of specific guidelines for the propagation of Colorado plants by seed.

Growth chamber studies were initiated in January 1979. After just two months of study several significant findings can be reported for green needlegrass (*Stipa viridula*), Utah sweetvetch, and green ephedra (*Ephedra viridis*). Each species is presently being tested in various seed mixtures at the Intensive Study Site. However, germination and emergence responses have not been consistently high. Green needlegrass is a cool-season species and should respond to favorable moisture conditions early in the growing season when temperatures are moderate. Growth chamber tests at the present time indicate that green needlegrass germinates best at 28°C and higher temperatures under dark conditions following one month of cold stratification. This response of green needlegrass to higher temperatures may be one explanation for its unpredictable nature in the field.

Utah sweetvetch has a hard seed coat and will not germinate unless scarified. Since this species has not been reported in the literature, information on seed physiology and germination characteristics will be established in this study. The simple fact that seed coat scarification is essential for germination may explain its low response in the field.

Green ephedra, a native shrub common in the oil shale regions of Colorado and Utah, has displayed an ability to successfully germinate at low temperatures. Two sources of seed were subjected to a cold stratification treatment (2-4°C) for one-, two-, and three-month periods to determine optimum stratification time prior to initiation of germination studies. No germination was reported after one month, but greater than 40 percent germination was reported after two months and 90 percent germination after three months. This response indicates that green ephedra is capable of germination in late winter or early spring if proper moisture and stratification conditions have been met. In fact, germination could occur so early in the growing season that snows or extremely cold weather would inhibit or prevent seedling establishment.

Summary

A three-volume publication entitled "Growing Colorado Plants from Seed--A State of the Art" (Volume 1--Shrubs, 2--Forbs, and 3--Grasses) will be completed in the fall of 1979. This publication will identify the status of current knowledge on propagation of ecologically important Colorado plants. Volume 1 has been submitted to the U.S. Forest Service for publication and should be available some time later this year.

Growth chamber experiments are underway to determine optimum germination and emergence requirements for three important revegetation species. This aspect of the study has just begun generating results and will make substantial contributions in the near future.

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ABSTRACTS OF PUBLICATIONS OR PRESENTATIONS

PLANT SUCCESSION ON DISTURBED LANDS ASSOCIATED WITH OIL SHALE DEVELOPMENT AS AFFECTED BY SPECIES MIXTURES, FERTILIZER, AND MULCH

Douglas Johnson* and **Edward F. Redente,** *Department of Range Science, Colorado State University, Fort Collins.*

A successional study was established in the fall of 1976 to determine rate and direction of plant community succession on surface disturbed soil as influenced by plant species mixtures, fertilizer, and mulch. A 2- hectare (4.9- acre) site located in a mid-elvation sagebrush community in the Piceance Basin was scraped of vegetation and then ripped to a depth of 30 cm (12 inches) with the use of a track-type dozer. The site was then fertilized, rototilled, seeded, and mulched in a split-block design with 108 subplots each measuring 9×18 m (29×59) feet). Treatments included (1) three fertilizer levels, (2) six species mixtures, and (3) two mulch treatments. The species mixtures used were (1) native grass, (2) introduced grass, (3) native grass-forb, (4) introduced grass-forb, (5) native grass-forb-shrub, and (6) combination native and introduced grass-forb-shrub. Subplots were monitored for plant emergence and establishment during the first growing season and survival and productivity of seeded and invading species during the second growing season. Plant mixture was the dominant factor affecting the development of early seral stages. Introduced grasses were more aggressive competitors and reduced the vigor of invading plants such as *Kochia, Salsola, Chenopodium, Bromus, Sphaeralcea, Phlox, Allium, Artemisia, Trifolium*, and *Haplopappus*. Invading species such as *Chenopodium, Salsola*, and *Kochia* were largely replaced the second year by *Bromus tectorum* and plants characteristic of more advanced seral stages owing to the increased competition of seeded plants. Introduced grasses also reduced the number and vigor of seeded forbs and shrubs, which resulted in the establishment of communities with less diversity. Native communities were more susceptible to invasion because of reduced competition from native species. Fertilizer did not have an effect on species composition but did influence vigor and overall biomass production. Mulch, however, had no influence on species composition, diversity, plant numbers, vigor, or productivity.

Presented at

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REVEGETATION TECHNIQUES ON DISTURBED LANDS ASSOCIATED WITH OIL SHALE DEVELOPMENT

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In the fall of 1976 a study was initiated to determine revegetation procedures for establishing productive and diverse ecosystems on lands disturbed by oil shale development in northwestern Colorado. It was regarded as imperative to accomplish this goal with a minimum input of scarce resources and to determine what these levels would be. An intensively disturbed plot was established by mixing topsoil and subsoil to a depth of 1 m (39) inches). The study was set up as a split block design with five levels of treatment including: (1) application of supplemental water, (2) replications, (3) seeding mixtures, (4) planting techniques, and (5) fertilization. Seeding mixtures included a diverse array of either native, introduced, or combination native-introduced grasses, forbs, and shrubs. Each mixture was planted with four techniques which were designed to facilitate establishment of a diverse plant community. Results following the second growing season have shown that shrub species can be established in combination with grasses and forbs by increasing the seeding rate of shrubs and decreasing the associated rate for the other life-forms. Seeding mixtures consisting of all introduced species were more effective than native or combination mixtures in providing rapid cover and soil protection. the introduced mixture also produced greater numbers of plants and higher amounts of biomass. Application of supplemental water resulted in the establishment of closed plant communities by the end of the second growing season, regardless of seed mixture or planting technique used. Fertilization significantly favored numbers of plants, production of biomass, and cover. Relative to adjacent native communities, revegetated sites were shown to reach higher levels of productivity within two years of seeding under certain combinations of treatments. Original levels of diversity, however, will not be reached until further successional processes alter the revegetated site.

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EARLY PLANT SUCCESSION ON SIMULATED OIL SHALE DISTURBANCES

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Successful stabilization and revegetation of spent oil shale material from surface retorting operations is of critical importance to the future development of oil shale in northwestern Colorado. Before spent shale can be disposed of in an environmentally safe manner, research must be conducted to determine proper disposal schemes and revegetation procedures. This study addresses these problems through the establishment of a 1.6-hectare test site utilizing 13,600 metric tons of Paraho retorted shale in several disposal schemes. Results have indicated that plant establishment on retorted shale, as a growth media, was not possible when soil amendments and irrigation were withheld. On disposal schemes with various depths of topsoil over retorted shale, results have shown greater plant vigor and productivity as the soil cover was increased in depth from 30 to 91 cm. Early successional trends have also indicated that diverse mixtures of introduced grasses, forbs, and shrubs approached a closed community more rapidly than native species mixtures. Because of the higher emergence, establishment and survival rate along with more rapid and vigorous growth, introduced species were able to reduce the entry of invading annuals at a significantly greater level than native species.

Presented at

Society for Range Management Annual Meeting
Casper, Wyoming
12-15 February 1979

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Title-Summary Form -- ASA-CSSA-SSSA 1979 Annual Meetings, 5-10 Aug., Ft. Collins, Colorado

1) Title:

The Effects of Reclamation Techniques on Successional Processes

2) Author(s)--indicate speaker with asterisk (*); see Prog. Reg. 3b

	Name	Membership class		Name	Membership Class
1st)	Edward F. Redente	---	3rd)	Walter J. Ruzzo	---
2nd)	Donald A. Klein	Active	4th)		

3) Summary (type single space; use back side for more space):

The establishment of diverse and self-sustaining ecosystems on disturbed oil shale lands in northwestern Colorado is being studied. Various seed mixtures, seeding techniques, fertilizers, and supplemental water are being tested to determine the best combinations for achieving successful reclamation with minimum inputs of scarce resources in the aboveground plant community and also changes in soil microbial types and activities. The coordinated effort of sampling and monitoring both environmental compartments has provided greater insight into the interpretation of treatment responses.

4) Give name and location of employer at the time the research was done:

Range Science Dept. and Microbiology Dept., Colorado State University, Fort Collins

5) Give mailing address and telephone number for author who will handle correspondence:

Title (Dr. Mr. Prof.) and name Department or section University or other organization Street No. or P.O. Box if needed City, State, Zip Code	Mr. Edward F. Redente Deparmtent of Range Science Colorado State University Fort Collins, Colorado 80523	Telephone no: (303) 491-6541 Alternate address:

6) Is paper volunteered or invited? volunteered

7) If invited, by what program chairman was it invited? Name ______
Society or Div. ______
If invited, how much time is desired (15, 30, 45 min.)? ______

8) Name of program chairman to whom Title-Summary was sent: R. C. Menzel
a) Preferred subject matter Division (Letter and Number): A-5
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American Society of Agronomy--Crop Science Society of America--Soil Science Society of America
Madison, Wisconsin 53711 USA

REVEGETATION OF RETORTED OIL SHALE MATERIAL:
A TOPSOIL DEPTH STUDY

by

E. F. Redente
and
W. J. Ruzzo

Successful stabilization and revegetation of retorted oil shale material from surface retorting operations is of critical importnace to the future development of oil shale in northwestern Colorado. A topsoil depth study is presently underway in the Piceance Basin to determine the optimum topsoil depth for revegetating retorted shale material. Approximately 13,600 metric tons of retorted shale were transported to the interior of the Piceance Basin from the Paraho retort at Anvil Points. Following transport and construction of a 1.6 hectare test plot various plant species mixtures were seeded and are presently being monitored to determine the effect that retorted shale properties, depth, and disposition have on plant establishment, survival, and long-term community succession. Instrumentation has also been installed to monitor the change in solubility and overall movement of salts, trace metals, and residual organics within the soil-shale profiles.

Submitted to EPA Oil Shale Sampling, Analysis and Quality Assurance Symposium. Denver, Colorado.
March 26, 27, 28, 1979.

ABSTRACT

Vories, Kimery C. 1978. Growing Colorado Plants from Seed: A State of the Art. Volume 1: Shrubs.

Information on the germination and establishment of most wildland shrubs from seed has become increasingly important with the increase in desire to reestablish self-supporting ecosystems on lands disturbed by human activity. The information available is highly variable as to its quality, quantity, and accessability. The purpose of this investigation is to incorporate existing germination and plant propagation information in an easily usable format for persons engaged in plant shrubs, native or naturalized to the State of Colorado. Information included is related to the (1) seed procurement, (2) pretreatment, (3) laboratory germination, and (4) cultural practices of 127 Colorado shrub species. Additional information includes: (1) 234 literature citations, (2) a list of the Colorado shrub species which have been evaluated by USDA Soil Conservation Service Plant Material Centers, (3) addresses of western United States Plant Materials Centers, (4) a list of the commercial suppliers of seed, seedlings, and transplants of Colorado shrubs, and (5) a list of the addresses of commercial suppliers of Colorado shrubs.

KEY WORDS: Germination, propagation, shrubs, Colorado seeds, field planting

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PUBLICATIONS IN PROGRESS

1. The Effect of Various Cultural Practices on the Revegetation of Lands Disturbed by Oil Shale Development

   Redente, Edward F., Ronald F. Jepson, and Linda Joyce

   Reclamation Review

2. Early Successional Patterns on Reseeded, Shallowly Disturbed Soils in the Piceance Basin, Colorado

   Redente, Edward F., Douglas E. Johnson, and Linda Joyce

   Reclamation Review

3. The Effect of Seed Mixture and Fertilizer Treatment on First-Year Invasion in a Sagebrush Community

   Redente, Edward F., Walter J. Ruzzo, and Linda Joyce

   Journal of Range Management

4. The Relationship between Microorganisms Activity and Early Successional Patterns on Intensively Disturbed Soils in a Sagebrush Community

   Redente, Edward F., Donald A. Klein, and F. Brent Reeves

   Reclamation Review

LONG-TERM FERTILITY MAINTENANCE ON LAND DRASTICALLY DISTURBED BY OIL SHALE DEVELOPMENT

Personnel: Dr. Burns R. Sabey, Professor
Dr. William A. Berg, Professor
Ms. Katherine Corwin, Graduate Research Assistant

OBJECTIVES

The objective of this subproject is to determine the long-term fertility requirements and methods of meeting these requirements of N- and P-deficient soil materials disturbed by oil shale development in northwestern Colorado. The following treatments are being investigated:

1. Adding low to moderate annual applications of inorganic fertilizer N over a four-year period,
2. Adding high rates of inorganic fertilizer N only at the initiation of the study to equal the summation of the rates in Treatment 1 after four years,
3. Adding high rates of N as in Treatment 2 plus sawdust at the initiation of the study,
4. Adding moderate to high rates of sewage sludge and sawdust at the initiation of the study,
5. Adding two rates of P with moderate and high rates of inorganic fertilizer N,
6. Adding no nitrogen fertilizer, and

7. Seeding native and introduced legumes in pure stands and in a native grass mixture with and without nitrogen fertilizer.

PROGRESS TO DATE

Long-Term Fertility Plot

The Long-Term Fertility Study examines adding nitrogen to the soil system from both inorganic and organic external sources and internally through N-fixation by legumes. As these methods are evaluated in light of the overall project's objectives, other aspects of maintaining adequate nitrogen in the soil will come under consideration. The first is economics. At present it is difficult to evaluate and compare the cost of adding N by these different methods. Another consideration in adding N to the soil is its long-term effectiveness which must be considered in the final evaluation. These aspects of the study are not directly measurable, but may well prove to be the deciding factors in the approach chosen to maintain N fertility in these soils.

The Long-Term Fertility Plots were constructed in the summer and fall of 1977. One set of plots was constructed on disturbed topsoil placed over 61 cm of Paraho retorted shale (Figure 38) while the second set of plots was established on disturbed subsoil (Figure 39). These two sets were prepared to compare nitrogen availability to plants in an N-deficient subsoil with a topsoil that has considerably more organic matter and thus more nitrogen available for plant growth.

A uniform application of 130 kg P/ha was broadcast over all plots except the N-P interaction plots. After the treatments were surface applied, they were worked into the soil by rototilling. The native seed

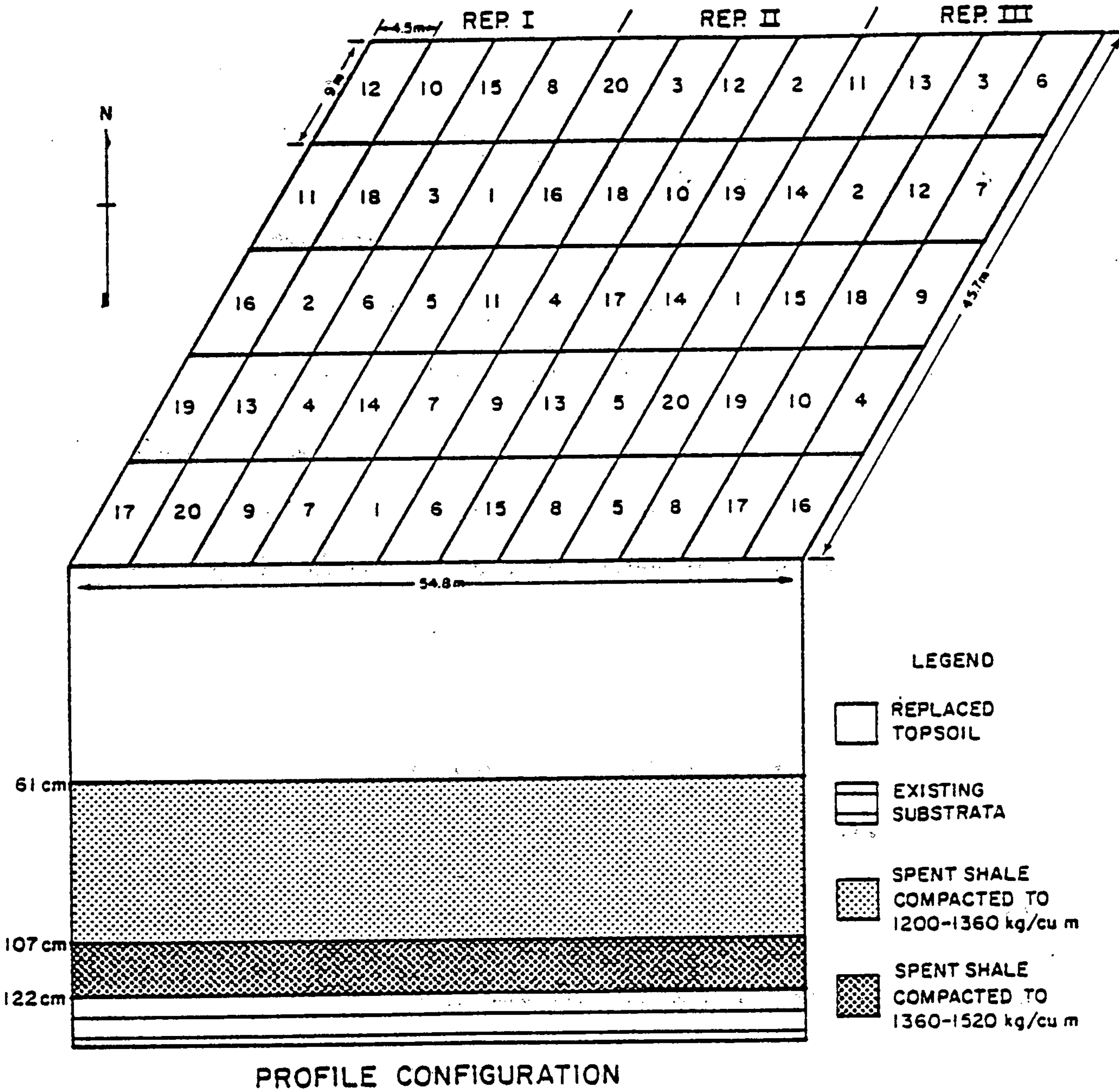


Figure 38. Profile configuration and experimental design for Long-Term Fertility Plots over retorted shale material.

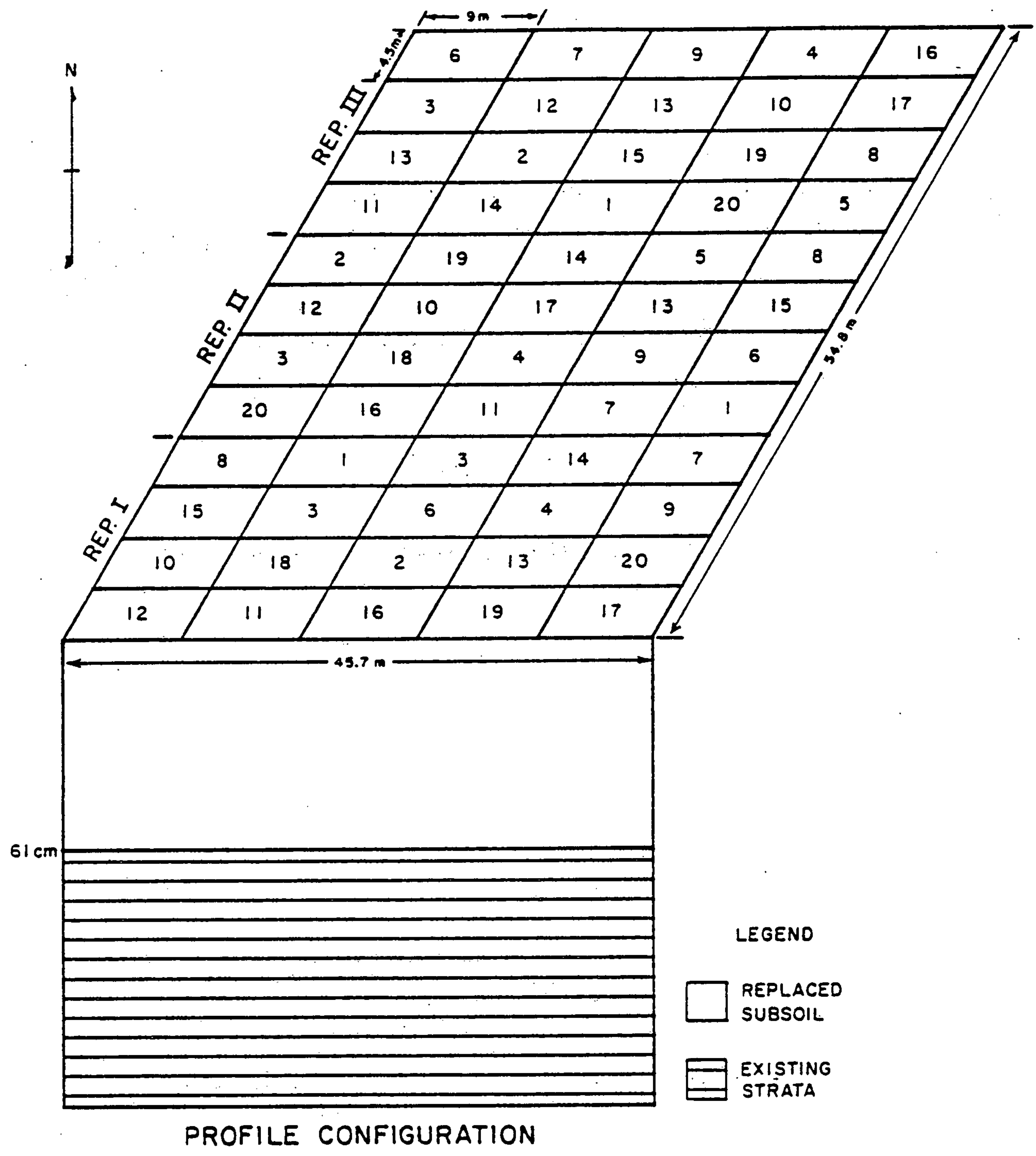


Figure 39. Profile configuration and experimental design for Long-Term Fertility Plots over subsoil material.

mixture used on the Retorted Shale Successional Plot was drill seeded on the treated plots in November 1977 (Table 11).

Favorable moisture conditions in the Piceance Basin in 1978 (the first growing season for the Long-Term Fertility Plots) resulted in good plant establishment on both the topsoil over retorted shale and the subsoil plot. However, both stands have some rows that were not seeded due to drill problems caused by inclement weather during the 1977 fall seeding operation. The individual rows that were not planted will be reseeded when weather permits in the coming growing season. Soil was sampled in each subplot in April 1978 and analyzed for total nitrogen, nitrate and ammonium nitrogen, plant available phosphorus, and salts. Plant growth was monitored twice during the first growing season to determine initial germination and survival levels. Plant responses measured were maximum height, density, cover, and biomass on six permanently-placed quadrats in each subplot. These responses as affected by fertility treatments are currently under study. Total nitrogen was analyzed in plant material clipped late in the growing season from three randomly-placed quadrats in each subplot and is being evaluated for efficiency of nitrogen use by the plants as affected by fertility treatments

The subsoil treated with sewage sludge contained substantially more invaders (mainly, *Salsola kali* and *Kochia* spp.) than the other subplots. In spite of these invaders, the native grass-forb-shrub mixture showed good establishment on these plots. These invaders were removed in August 1978 before dropping seed by cutting the plants off at ground level and disposing of them off site. This was done to minimize weed competition in the reseeded rows during the coming growing season.

Table 11. Species mixture and seeding rates used on Long-Term Fertility Plots.

	Common Name	Scientific Name	Seeding Rate PLS (kg/ha)
1.	Rosana western wheatgrass	*Agropyron smithii*	1.12
2.	Sodar streambank wheatgrass	*A. riparium*	1.12
3.	Bearded bluebunch wheatgrass	*A. spicatum*	1.12
4.	Indian ricegrass	*Oryzopsis hymenoides*	1.12
5.	Green needlegrass	*Stipa viridula*	1.12
6.	Durar hard fescur	*Festuca ovina duriuscula*	0.56
7.	Shermans big bluegrass	*Poa ampla*	1.12
8.	Alkali sacaton	*Sporobolus airoides*	0.56
9.	Globemallow	*Sphaeralcea munroana*	0.56
10.	Utah sweetvetch	*Hedysarum boreale utahensis*	1.12
11.	Palmer penstemon	*Penstemon palmeri*	0.56
12.	Stansbury cliffrose	*Cowania mexicana stansburiana*	2.24
13.	Green ephedra	*Ephedra viridis*	1.12
14.	Fourwing saltbush	*Atriplex canescens*	1.12
15.	Winterfat	*Ceratoides lanata*	1.12
16.	Antelope bitterbrush	*Purshia tridentata*	1.12

Annual fertility treatments were applied in the fall of 1978 in preparation for the second growing season. Plant monitoring on the permanent quadrats will continue during successive growing seasons. After the second growing season plant succession as a function of fertility will be included in the data analysis.

Since this study was designed primarily as a long-term fertility study, meaningful data interpretation is not possible after the first growing season. First-year observations and measurements will be incorporated with succeeding years' data and interpreted over time with respect to long-term fertility maintenance on nitrogen deficient soils in the Piceance Basin. The conclusions based on the first year's results are tentative.

Results

Total N, NO_3-N, and NH_4-N in the Soil Samples

A one-way analysis of variance was used to determine if any differences existed in the soil nitrogen content after the spring 1978 sampling. If significant differences existed, an LSD was made at the 10 percent level.

Table 12 indicates that the values for total nitrogen in the soils ranged from 0.07 to 0.19 percent and 0.03 to 0.13 percent in the topsoil and subsoil subplots, respectively. There was a significant increase in total N content of the soil resulting from the addition of sewage sludge as well as from the highest rate of inorganic N added with and without wood wastes to both the topsoil and subsoil plots. All other treatments were not different than the control at the 10 percent level of significance.

Table 12. Results of soil analyses for total nitrogen, nitrate nitrogen, and ammonium nitrogen on 1978 soil samples.

No.	Rate of Treatment (kg/ha)	Total Nitrogen[1] (%)		Nitrate Nitrogen[1] (ppm)		Ammonium Nitrogen[1] (ppm)	
		Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
	Annual rates of N[2]						
1	56 N	0.075	0.041	1.83	1.33	10.33	13.33
2	112 N	0.075	0.043	2.33	1.00	6.33	15.33
3	224 N	0.076	0.053[3]	2.00	4.67	19.67[3]	43.67
4	448 N	0.072	0.040	5.33	1.33	7.33	30.33
	Single rates of N						
5	224 N	0.078	0.040	3.33	2.67	8.00	23.00
6	448 N	0.073	0.047	5.33	3.33	6.00	32.33
7	896 N	0.074	0.044	15.33[3]	3.00	12.33	45.67
8	1,793 N	0.084	0.059[3]	40.00[3]	2.67	19.67[3]	58.33[3]
	Single rates of N as above plus wood wastes (ww)						
9	224 N + 11,120 ww	0.074	0.037	1.00	1.67	6.67	19.67
10	448 N + 22,400 ww	0.075	0.041	2.67	2.33	9.00	26.67
11	896 N + 44,800 ww	0.075	0.048	9.00	3.00	9.67	44.67
12	1,793 N + 89,700 ww	0.88[3]	0.066[3]	89.67[3]	11.33[3]	29.67[3]	98.67[3]

Table 12.--Continued

No.	Rate of Treatment (kg/ha)	Total Nitrogen[1] (%)		Nitrate Nitrogen[1] (ppm)		Ammonium Nitrogen[1] (ppm)	
		Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
	Sewage sludge (ss) + ww						
13	56,000 ss + 0 ww	0.103[3]	0.063[3]	5.33	1.33	5.00	24.33
14	112,000 ss + 22,400 ww	0.146[3]	0.130	4.67	2.67	5.67	30.33
15	224,000 ss + 44,800 ww	0.188[3]	0.123	8.00	3.33	3.00	24.33
	P and N interaction						
16	112 N + 56 P[4]	0.072	0.042	2.00	1.33	1.33	16.67
17	896 N + 56 P	0.081	0.050	11.67	1.33	6.67	60.00[3]
18	112 N + 192 P	0.072	0.036	1.33	2.17	1.67	17.33
19	896 N + 192 P	0.078	0.049	6.33	1.17	6.33	57.00[3]
	Control						
20	0 N + 130 P	0.075	0.035	1.33	1.00	3.33	12.67
	LSD ($P \leq 0.10$)	0.010	0.017	12.27	3.86	10.90	22.80

[1]Average of three replications.

[2]N applied as NH_4NO_3.

[3]Significantly different from the Control Treatment (20) at the 10 percent level.

[4]P applied as triple super phosphate.

There was considerably less nitrate nitrogen in the subsoil than in the topsoil as expected in most treatments. The first three wood waste treatments (9, 10, and 11) apparently immobilized nitrate since there was less nitrate in these plots than in comparable plots (5, 6, and 7) without wood wastes. Nitrate content in the high N treatments (7, 8, and 12) of the topsoil plots and number 12 of the subsoil plots were significantly higher than the controls at the 10 percent level.

The ammonium nitrogen content of the subsoil subplots generally was higher than the topsoil plots. This may have been due to a higher nitrifying bacterial population in the topsoil than in the subsoil. Measurements of nitrifier populations in the subplots by Dr. Klein of the Department of Microbiology (CSU) next summer could verify this hypothesis. Only topsoil treatments 3, 8, and 12 were significantly different than the control. The high N treatments were 8 and 12. Treatment 3 was a relatively low N application subplot. The reason for higher ammonium content in this subplot than all the other subplots is not known. Subsoil treatments 8, 12, and 19 were all significantly higher in ammonium nitrogen than the control and were all high N treatments. There was more ammonium nitrogen in the high N with wood wastes than the high N without wood wastes for both topsoil and subsoil subplots.

An evaluation of the total nitrogen added as inorganic N or as sewage sludge compared to that accounted for in the soil and plant analyses shows that much of the N was lost from the soil by leaching, denitrification, plant uptake, or was not measured due to potential sampling and analytical errors. Since the precipitation in the Picenace Basin is relatively low and the soil was not saturated for a long period of time, leaching and denitrification do not seem likely. However, we are going to sample the soil (in the spring of 1979) to at least 61 cm to determine if nitrates are leaching.

Total N in the Grasses

Four high nitrogen treatments (11, 12, 17, and 18) in the subsoil subplots showed a higher N content (Table 13) in harvested grasses than in grasses from the control. In topsoil subplots 5 and 13 the grasses utilized lower quantities of N than in the control. A logical explanation of this is not apparent at this time. There were no significant differences in total N in the grasses due to any of the other treatments on the topsoil.

The amount of total N taken up by the grasses falls far short of accounting for all the N added to the plants by sludge or fertilizer. Large quantities of the N in the system have been lost or are unaccounted for. This problem in part may be due to the difficulty of analyzing different forms of nitrogen in the soil.

Vegetation Evaluation

A subjective evaluation of plant growth (Table 13) due to the various fertility treatments was made on each subplot early and late in the 1978 growing season. It consisted of a visual observation estimating a composite of plant frequency, dnesity, and cover. Values were assigned ranging from one to five with five being the best plant response and one being the poorest response. Although a valid statistical analysis is not feasible, it appears that plant growth in topsoil treatment 12 was less than plant growth in the control. This may be caused by over fertilization and a high wood waste application. Topsoil treatments 5 and 19 (high N and P) were evaluated as better than the control (Table 13). The

Table 13. Total nitrogen in grasses, plant yields, and subjective evaluations on 1978 plant samples.

No.	Rate of Treatment (kg/ha)	Total Nitrogen in Grasses (%)		Dry Weight Yield[1] (kg/ha)		Subjective Evaluation[2,3]	
		Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
	Annual rates of N[4]						
1	56 N	2.17	1.50	854	556	2.75	3.25
2	112 N	2.17	1.40	326	748	2.40	2.92
3	224 N	2.27	1.80	387	413	1.83	2.58
4	448 N	2.10	1.53	632	620	2.67	2.67
	Single rates of N						
5	224 N	1.95[5]	1.90[5]	720	512	3.50	2.67
6	448 N	2.13	1.93[5]	784	338	2.33	2.67
7	896 N	2.30	1.93[5]	420	214	2.83	1.75
8	1,793 N	2.25	1.90[5]	392	171	1.67	1.42
	Single rates of N as above plus wood wastes (ww)						
9	224 N + 11,120 ww	2.23	1.50	612	532	2.33	2.75
10	448 N + 22,400 ww	2.27	1.83	308	728	2.17	2.83
11	896 N + 44,800 ww	2.45	2.00[5]	240	436	1.75	2.17
12	1,793 N + 89,700 ww	--	2.43[5]	173	167	1.08	1.42

Table 13.--Continued

No.	Rate of Treatment (kg/ha)	Total Nitrogen in Grasses (%)		Dry Weight Yield[1] (kg/ha)		Subjective Evaluation[2,3]	
		Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
	Sewage sludge (ss) + ww						
13	56,000 ss + 0 ww	1.97[5]	1.68	872	696	3.00	3.75
14	112,000 ss + 22,400 ww	2.10	1.30	594	764	3.00	3.33
15	224,000 ss + 44,800 ww	2.23	1.67	892	485	2.50	3.67
	P and N interaction						
16	112 N + 56 P[6]	2.10	1.75	640	464	3.00	2.50
17	896 N + 56 P	2.23	2.57[5]	535	245	2.67	1.58
18	112 N + 192 P	2.27	1.40	428	679	2.17	3.75
19	896 N + 192 P	2.13	2.03[5]	820	370	3.58	1.85
	Control						
20	0 N + 130 P	2.25	1.53	416	648	2.33	2.75
	LSD ($P \leq 0.10$)	0.24	0.36	n.s.	345		

[1]Average of three subsamples in three replicates.

[2]Average of three replicates over two sampling times.

[3]Evaluated on the following criteria: (1) no growth or scattered, single plants; (2) sparse stand; (3) patchy stand or rows not uniform; (4) fair stand; (5) good stand plus high vigor.

[4]N applied as NH_4NO_3

[5]Significantly different from the control (20) at the 10 percent level.

[6]P applied as triple super phosphate.

three treatments involving sludge appeared to have better growth than the control. These observations roughly parallel the herbage data for sludge treated soils (Table 13).

The subsoil plots showed different growth trends than the plots with topsoil over spent shale. The sludge treatments on the subsoil plots resulted in the highest evaluations. In addition, treatments 1, 2, 13, 14, 15, and 18 also showed a positive response when compared with the control. Treatments 3, 6, 7, 11, 12, 16, 17, and 19 were all evaluated lower than the control. Most of these treatments included excessively high rates of N with the exception of treatments 3 and 16.

An evaluation of the effect of the wood residues on plant density and maximum height was made on treatments (5, 6, 7, and 8) with low to high rates of N and on treatments (8, 9, 10, and 11) with the same low to high rates of N plus wood wastes using a two-way analysis of variance. In these treatments it was evident that the lowest plant density and height occurred on the topsoil plots treated with the highest levels of nitrogen and wood waste (treatments 11 and 12). It appears that the wood residue is causing a decrease in plant growth because the height and density on the subplots with high nitrogen without wood residues (7 and 8) are not significantly different than the control at the 5 percent level. The reason for the detrimental effects of the wood residues may be due to the phytotoxic effect of compounds they contain, the effect the wood residues have on the moisture regime, or the immobilization of nitrogen due to the high C/N ratio. The latter is unlikely because the high levels of nitrogen added initially provide more nitrogen than is needed to overcome any effects of immobilization. These hypotheses will be investigated during the coming growing season.

Legume Plot

The legume study has the same basic objectives as the fertility treatments on topsoil over spent shale and subsoil, but takes a different approach to the problem of adding nitrogen to a deficient soil through natural plant fixation. According to Woodmansee et al. (1979) in a western grassland situation,

> Nitrogen availability is considered to be largely controlled by the cycling of N within living plants and by mineralization of easily decomposable organic matter. Only 4 to 13 percent of the N requirements of plants may be supplied from external sources.

The feasibility of adding N from within the living system is therefore of major interest.

A viable and balanced soil microbial population is recognized as important in establishing and maintaining adequate N fertility in disturbed soil. This aspect of the soil environment is closely tied to this study as the growth and development of bacteria from the genus *Rhizobium* will determine the effectiveness of the legumes in fixing atmospheric nitrogen.

The Legume Plot was established in 1978 in conjunction with the Long-Term Fertility Plots. It is a randomized block design with 14 species treatments, 3 levels of fertility, 2 soils, and 3 replications (Figures 40 and 41).

The test plot 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 61 cm and the grade leveled from east to west. Soil was replaced so that each replicate contained both a subsoil and a topsoil treatment in a random order.

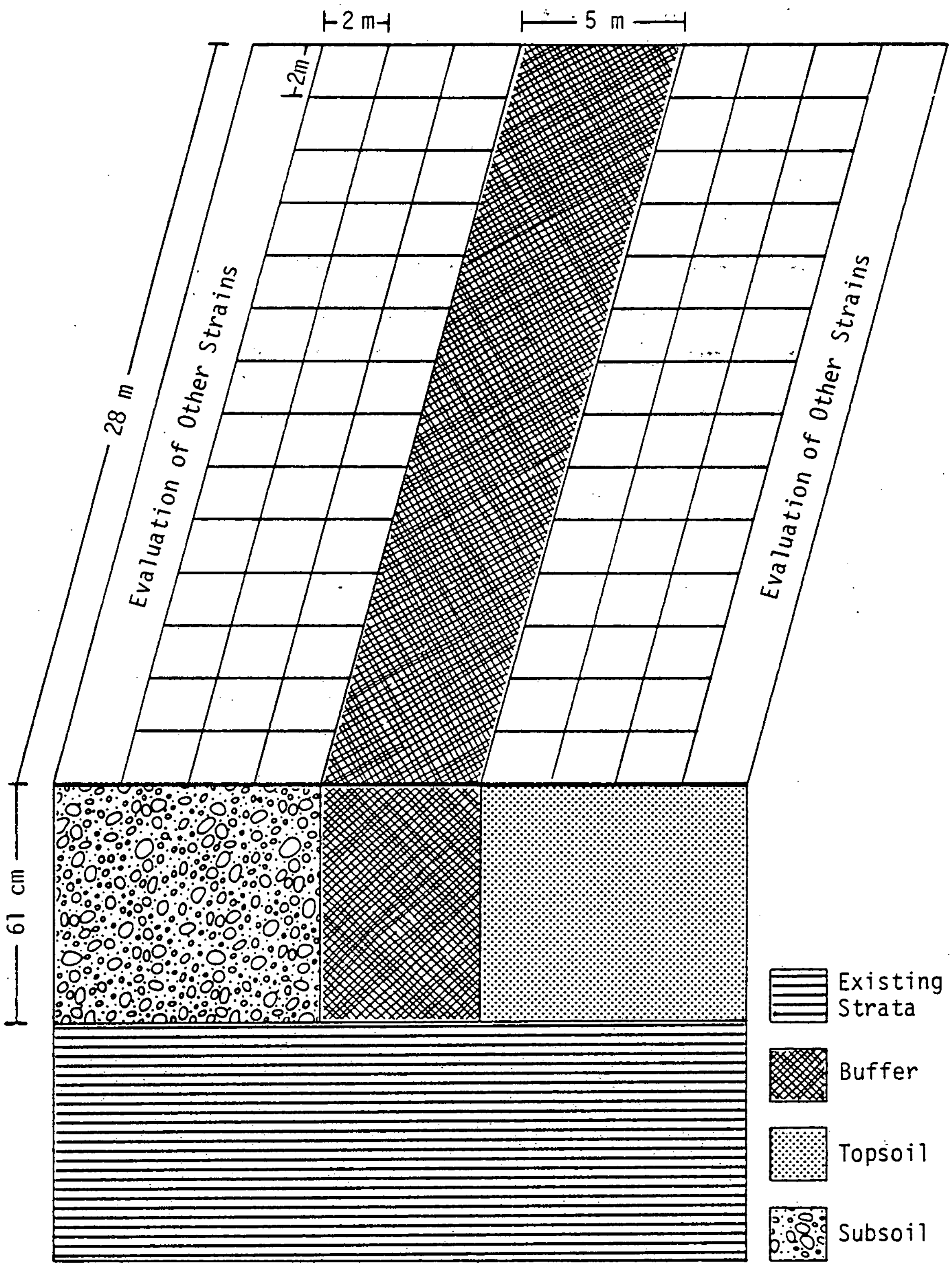
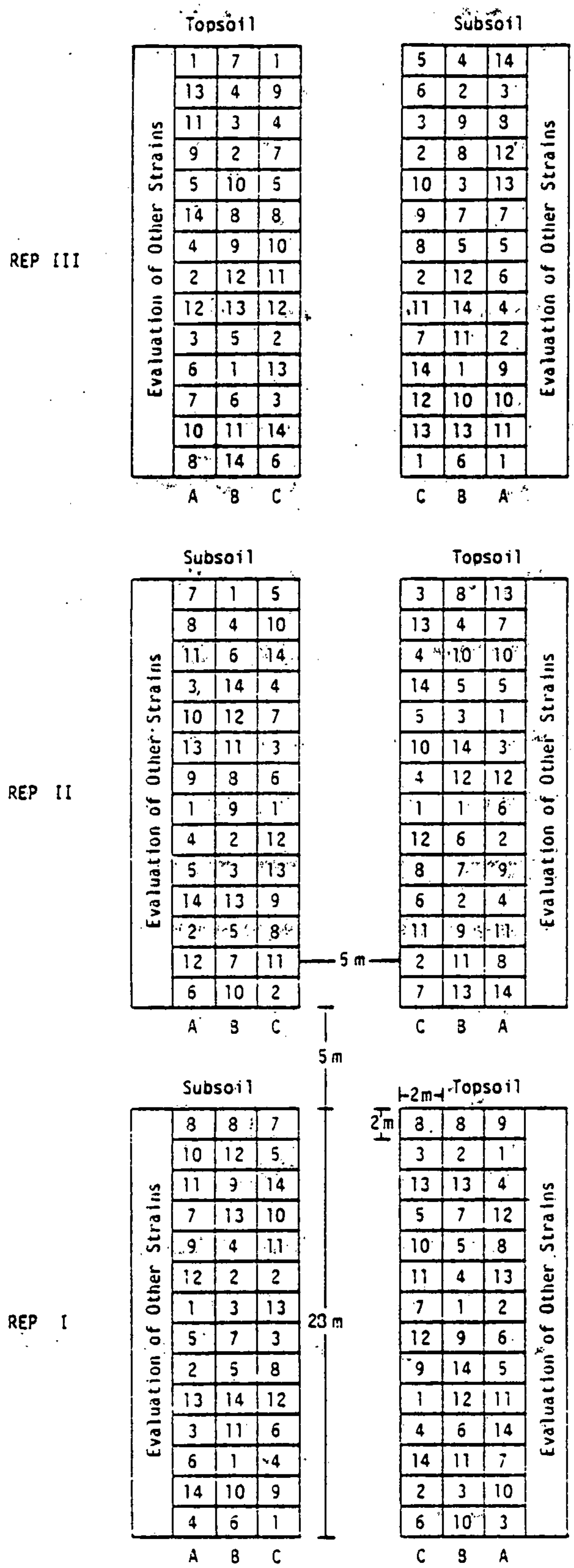


Figure 40. Profile configuration of one replication (REP I) from the Legume Study.



N

<u>Species Seeded</u>

1 = Utah sweetvetch
2 = Utah sweetvetch plus inoculum
3 = Penngift crownvetch
4 = Lutana cicer milkvetch
5 = Eski sainfoin
6 = Ladak alfalfa
7 = Utah sweetvetch and grass
8 = Utah 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

<u>Fertilizer Rate</u>

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

Figure 41. Field map of the Legume Plot.

A 5 m border was left between each soil treatment to insure a homogenous soil type.

After construction was completed, the soil was sampled in each panel to a depth of 30 cm with a soil probe. The results of analysis for total N, plant available P, pH, and salts are listed in Table 14. Total subsoil nitrogen is lower than topsoil N, as expected, and there are no large differences in soils between replicates. Salt levels are higher in subsoil, which may be a problem for the growth of some legumes with a low salt tolerance. Native P was very low.

To correct the phosphorus deficiency, the plot was fertilized at 190 kg P/ha with triple super phosphate in September 1978. 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 approximately six weeks after fertilization and seedebed preparation to allow the fluffy soil to settle and form a crust. Table 15 lists the species planted, the seed source, and the inoculum treatment. The legumes were broadcast seeded at a rate of 430 seeds/m^2 (40 seeds/ft^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 mixture (Table 16) was seeded at the same density, divided equally between legumes and grasses. Portable wooden frames the dimensions of each subplot (2x2x0.03 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

Table 14. Results of soil tests following legume plot construction.

		Topsoil				Subsoil			
Replication	Fertilizer	pH	Conductivity	$NaCO_3$, ppmP[3]	% Total N	pH	Conductivity	$NaCO_3$, ppmP[3]	% Total N
I	AA	8.3	0.6	3	0.076	8.3	1.9	3	0.058
	A	8.2	0.8	4	0.079	8.1	1.4	4	0.053
	B	8.0	0.6	4	0.080	8.1	1.7	3	0.058
	C	8.0	0.5	4	0.079	8.0	1.1	2	0.058
II	AA	8.0	0.6	3	0.076	8.2	1.2	2	0.060
	A	8.1	0.6	4	0.077	8.0	1.4	2	0.058
	B	7.9	0.6	4	0.076	8.1	1.4	2	0.054
	C	8.2	0.5	4	0.078	8.0	1.0	2	0.059
III	AA	8.0	0.6	3	0.078	8.2	1.0	3	0.063
	A	8.1	0.5	6	0.076	8.3	0.8	4	0.066
	B	8.0	0.7	4	0.080	8.0	0.7	4	0.066
	C	7.9	0.5	4	0.079	8.0	0.9	3	0.071

Table 15. Species planted, seed source, and type of inoculum used on the Legume Plot.

Treatment Number	Species Seeded and Source	Inoculum
1	*Hedysarum boreale utahensis* Utah sweetvetch, Stewart and Sons	None
2	*Hedysarum boreale utahensis* Utah sweetvetch, Stewart and Sons	Native soil
3	*Coronilla varia* Penngift crownvetch, Northrup King	Commercial
4	*Astragalus cicer* Lutana cicer milkvetch, Northrup King	Commercial
5	*Onobrychis viciaefolia* Eski sainfoin, Northrup King	Commercial
6	*Medicago sativa* Ladak alfalfa, Northrup King	Commercial
7	No. 1 plus native grass mixture[1]	None
8	No. 1 plus native grass mixture	Native soil
9	No. 3 plus native grass mixture	Commercial
10	No. 4 plus native grass mixture	Commercial
11	No. 5 plus native grass mixture	Commercial
12	No. 6 plus native grass mixture	Commercial
13	Native grass mixture	None
14	Native grass mixture	None

[1]Refer to Table 16 for a list of the grasses in the native grass mixture.

Table 16. List of native grasses used in grass-legume seed mixture.

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

row seeded (Table 17). These species will be studied for adaptation to the Piceance Basin area in conjunction with Dr. Cuany of the Department of Agronomy. Dr. Klein will conduct acetylene reduction analysis to determine the N_2 fixing ability of the legumes. Roots will also be examined for nodulation.

Plants will be sampled in late summer and analyzed for total nitrogen. Soil will be sampled annually and analyzed for total N, pH, salts, and ammonium and nitrate nitrogen to determine the effect of legumes on the nitrogen content of the soil.

Summary

During the first growing season the native species mixture established a good stand on most of the fertility treatments, with the exceptions of rows that were missed in the seeding operation. Second-year data should provide greater insight as a more uniform stand is established. The topsoil and subsoil plots are affected differently to N fertilization, as seen by comparing ammonium nitrate contents of each soil. This may be due to differences in the soil microbial population which will be monitored beginning in the spring of 1979. Some N has been lost from the system and this may be a problem in determining the efficiency of nitrogen use by plants. Due to the nature of this study in comparing initial application of N fertilizer with annually applied treatments, publishable information is not expected until data from subsequent growing seasons are obtained.

Table 17. Legumes and seed sources in adaptation and N_2-fixation study.

Species	Major Plots (broadcast)	Minor Strains (in rows)
Utah sweetvetch	Stewart's[1]	Stewart's, ISS, E/ISS, pipeline
Alfalfa	Ladak	Ladak
Cicer milkvetch	Lutana	Lutana, C-4, Dotzenko, Strohman, Sugarbeet-2, Wellington, 20-15, Penngift, Emeral, Chemmy
Sainfoin	Eski	Eski, Melrose, Remont
Lupine	--	031, 142, 225, 232
Purple prairieclover	--	Kaneb
Swainson's pea	--	San Luis Valley

[1]With and without local soil for rhizobia inoculum. All others are preinoculated with the most appropriate inoculum.

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SUITABILITY OF NATIVE SHRUBS AND FORBS FOR SHRUBLAND RESTORATION DESIGN: THEIR POSSIBLE GENETIC VARIABILITY AND COMMUNITY-HABITAT RELATIONSHIPS

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Dr. R. L. Dix, Professor
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OBJECTIVES

This study has two parts: (1) to describe and interpret the naturally occurring, ecological-genetic variation of some important species in relation to the environments of different sites where they are found and (2) to describe and interpret the naturally occurring phyto-sociological structure in relation to its placement on the landscape. The first will contribute to final recommendations concerning which population characteristics to emphasize in species mixtures for individual sites. The second will provide the essential vegetation structure useful for reclamation design where the goal is reestablishment of stable and diverse communities similar to pre-mining conditions.

PROGRESS TO DATE

Part One--Ecotype Study

The study of variation among populations of a species derives most of its information from plant responses in a common garden, now established at the Intensive Study Site with shrub, grass, and forb species. Plant responses in a common environment can be partitioned into those having a genetic basis for differences and those due to plasticity of expression of a common genotype. Attributes which differ genetically among populations of a species and which may be closely related to success on specific sites include phenology, differential resource investment in height, leaf and reproductive growth, drought modifications, and juvenile success. Measurements on all but the last have begun in the garden, and evaluation of the latter has begun in growth chamber experiments.

The actual hypotheses tested here have the form that there are no genetic differences between populations from different environments for these attributes. Rejection indicates that there are such differences. Correlation of these differences with differences in the native environments and interpretation in terms of environmental selection then becomes the basis for matching different ecotypes as reclamation materials with the different sites to be reclaimed.

Methods--Field

Populations of six shrub, two grass, and one forb species were transplanted into a common garden (0.25 ha) at the Intensive Study Site in the Piceance Basin. Species are represented by three (*Atriplex canescens*) to 25 (*Amelanchier alnifolia/utahensis*)[1] populations--with mostly eight individuals from each population. Each species is represented by different populations within the Piceance Basin and from other areas of Colorado and southern Wyoming (Table 18).

[1]Taxonomic gradation have led us to treat these taxa as one entity.

Table 18. Number of populations and individuals in the common ecotype garden at the Intensive Study Site.

Species	Number of Populations			Number of Individuals Per Population
	Local (Piceance Basin)	Regional	Total	
Amelanchier alnifolia/utahensis	20	5	25	8
Atriplex canescens	2	1	3	10
Ceratoides (Eurotia) lanata	6	1	7	8
Cercocarpus montanus	4	5	9	6
Purshia tridentata	4	2	6	6
Symphoricarpos oreophilus	15	5	20	4-8
Koeleria cristata	5	3	8	8
Oryzopsis hymenoides	11	4	15	6
Sphaeralcea coccinea	7	2	9	8
TOTAL	74	28	102	

After initial establishment as rooted cuttings, transplants from the field, or seedlings the plants to be put in the garden were maintained in a lathhouse a Colorado State University. Each species was located in its own block in the garden, and all individuals were randomly placed in centers within the block (Figure 42).

Individual plants were given two liters of water and shaded throughout the summer by a shingle on the southwest side. Watering was done by gravity-fed, hand-held garden hose running from a 1,700 gallon tank located near the garden. To aid establishment plants were watered twice a week and later once a week with one liter per watering. Because of different requirements, watering of some species (e.g., *Amelanchier* and *Symphoricarpos*) continued to the end of August, 1978, while only three or four waterings were required by others (e.g., *Atriplex* and *Ceratoides*). Water will be added during the coming season only if necessary to keep the plants alive, and then all populations of a species will continue to be treated alike.

Protection from small mammals was provided at first by individual wire mesh cages staked around each plant. Later, a four foot hardware cloth fence (with two feet buried) was placed around the entire garden, and the cages were removed. Weeding between plants was easily done by hoeing.

After establishment each plant of the shrub and forb populations was measured or rated at least once for various characteristics which included leaf length and width, plant height, number of stems and leaves, phenological condition, and vigor (Table 19). It requires about three minutes per shrub plant for two people to measure and record leaf length and width of ten leaves per plant, number of leaves and stems per plant,

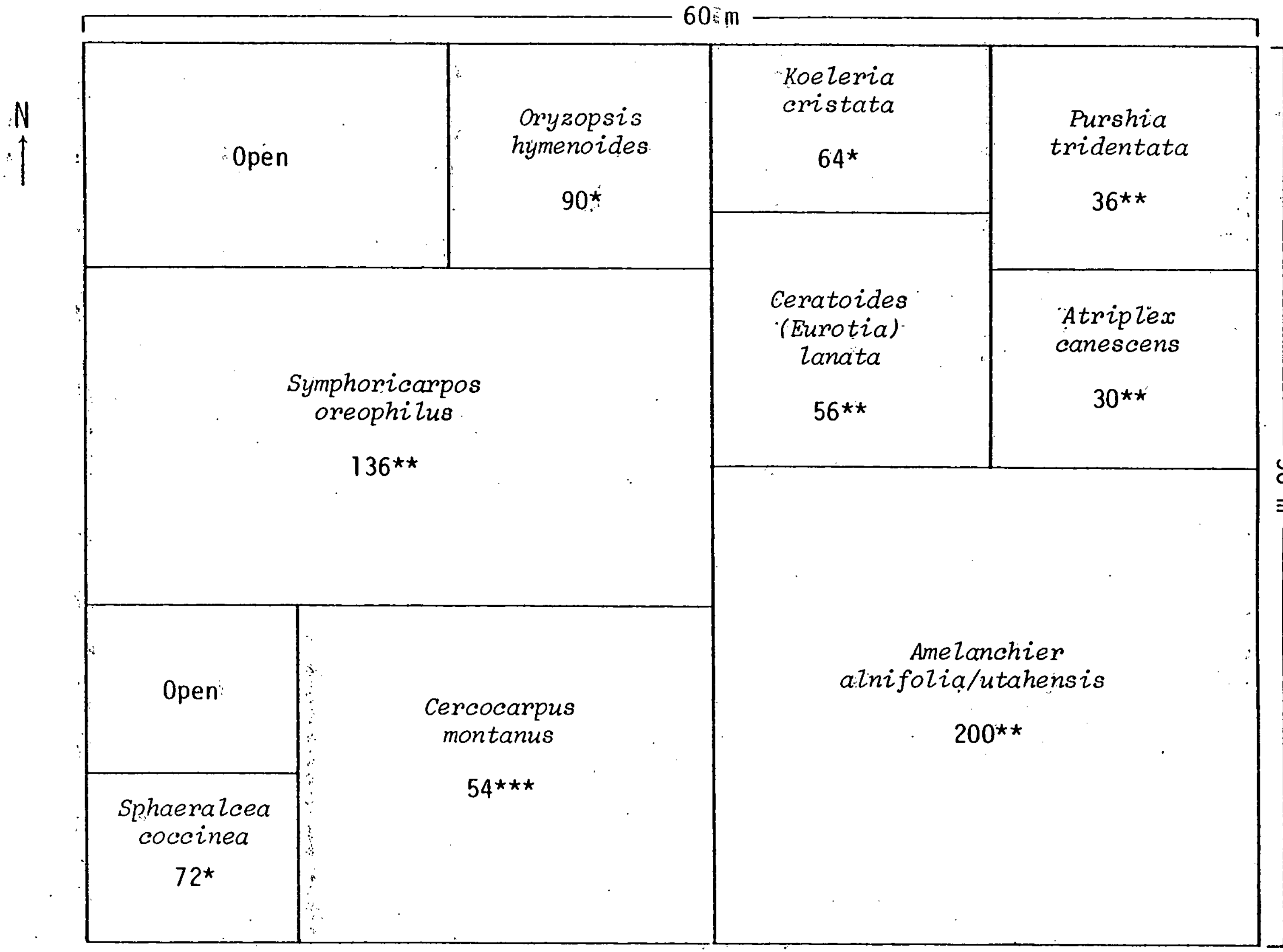

Figure 42. Map of common garden at the Intensive Study Site. (Shown is the total number of plants of each species on * 1 m centers, ** 1.5 m centers, or *** 2 m centers.)

Table 19. Characteristics for baseline measurements scored for every individual in the ecotype garden.[1]

Species	Leaf Length	Leaf Width	Number of Leaves	Number of Stems	Plant Height	Phenology
Amelanchier alnifolia/utahensis	x	x	x	x	x	
Atriplex canescens	xx	xx		xx	xx	x
Ceratoides (Eurotia) lanata				xx	xx	x
Cercocarpus montanus	x	x		x	x	
Purshia tridentata	x	x		x	x	
Symphoricarpos oreophilus	x	x	x	x	x	
Sphaeralcea coccinea	x	x	x			x

[1]Two marks mean measurements were taken at two different times, approximately one month apart. Leaf length and width were measured for ten leaves per plant to the nearest mm. Plant height was measured in mm. Phenology was scored as vegetative only, flowering or fruiting.

and plant height. This extends, for example, to ten hours each for two people to record these characteristics for the individuals of *Amelanchier* in the garden.

For analysis the length and width of leaves will be combined for additional understanding. Their product provides an index of leaf area and of the amount of surface exposed for evaportranspiration and devoted to photosynthesis, and dividing the length by the width gives an index of leaf shape. All of this information will provide the basis against which to measure data to be collected this next growing season.

Since all individuals for each species were subject to common treatment, the initial data may be a first indication of ecotypic differences among populations of a species.

The environment of each population collection has been characterized by 16 measures. These are listed and defined in Table 20.

Methods--Analysis

The methods of analysis fall into two groups. First, as in most ecotype garden studies, analysis of variance followed by a multiple range test is used to find out if there are statistically significant differences among the populations and to locate the differences. The differences can then be compared with differences in the original environments of the populations by correlation and regression. However, since our purpose goes beyond this in application to reclamation, a second kind of analysis is called for.

Basically, our intent is to find the interrelationships of three different and large sets of variables. These are the population variables measured in the garden (including sequential measurement of some of

Table 20. Environmental characteristics evaluated for each ecotype collection site.

Variable Number	Characteristics and Scalars
1	Elevation, m
2	Topographic position, scaled 1-5, upland to valley bottom
3	Percent slope, measured in field
4	Azimuth scalar, NE = 1, SW = 0
5	Annual precipitation, cm[1]
6	Annual temperature, °C[1]
7	Warm season ppt, cm/April-September[1]
8	Percent of ppt occurring in warm season[1]
9	Growing season length[1]
10	Latitude, °N
11	Longitude, °W
12	Solar horizontal equivalent latitude, °N[2]
13	Solar horizonal equivalent longitude, °W[2]
14	Radiation index[2]
15	Total annual potential insolation[2]
16	Total potential insolation June 22[2]

[1]Weather information for sites within the Piceance Basin are estimated from water balance equations developed for that area by Wymore (1974). Nearest weather station records provide the values for all other sites.

[2]From tables in Frank and Lee (1966).

them), the environmental variables characterizing the original collection site, and those characterizing the community. Multivariate methods are suggested for this kind of analysis since they can be used to

1. Simplify large sets of data,
2. Generate hypotheses concerning interrelations of the variables,
3. Give correlations between two or more sets of data, and
4. Generate new measures of indices for populations which efficiently summarize multivariate variation.

Biologically these features will aid interpretation of the ecotype garden study, respectively, by

1. Giving a simple structure of the intercorrelations of the biological variables measured,
2. Suggesting hypotheses concerning, for example, the trade-offs between reproductive and vegetative growth or between growth in size versus production of phytosynthetic surface,
3. Giving, for example, a way to see how the variation from ecotype to ecotype corresponds with structural variation from community to community, and
4. Assigning to the different ecotypes values indicating, for example, their relative adaptation to different kinds of sites.

Initial analyses of some of the garden and environmental measurements using, for example, principal component analysis, factor analysis, or canonical correlation analysis will be reported on below. Since the data collected to date in the garden represent only a fraction of the data anticipated, the presentation of results will be uneven but illustrative of the different kinds of analyses to be undertaken. For the full data set all appropriate analyses will be carried out.

Results

The discussion of results here has two purposes. First, to show that even the preliminary information we have suggests that there are ecotype differences within species of *Amelanchier, Purshia, Symphoricarpos,* and *Cercocarpus*. Second, to briefly indicate some methods of data analysis that will show how discovery and interpretation of ecotypic variation can be applied to reclamation.

Amelanchier alnifolia/utahensis

Ten of the 25 populations of *Amelanchier* were grown from seed and transplanted as six-month-old seedlings. All but two of these originated in the Piceance Basin. Measurements of leaf length and width of two leaves of each eight individuals showed significant differences among the ten populations for these characteristics (Table 21).

In order to locate the differences, Duncan's multiple range test was performed. The results indicate a gradient of genetic differences with significance (.05 level) absent from numerically close populations but present for more distant populations. If further data and analyses bear out these results, then genetic differentiation would be indicated.

Ecotypic (or ecoclinal) differences need to be interpreted in terms of the environment which selects and maintains the population differences. Our full approach will include, among other techniques, regression analysis of ecotypic differences with differences in the original environments of the plant populations. In this report we will present some results of correlation analysis. Table 22 gives the correlation coefficients (r) between leaf length and width, and these values with various environmental properties. No significance testing was done since the values were calculated using population means rather than raw data. The

Table 21. ANOVA tables of ten populations of *Amelanchier alnifolia/utahensis* for leaf length and leaf width.

	SS	DF	MS	F	Significant at .005
Leaf length					
Population	1335.3	9	148.37	6.13	yes
Residual	1695.6	70	24.21		
Total	3030.9	79			
Leaf width					
Population	807.7	9	89.7	7.29	yes
Residual	861.6	70	12.3		
Total	1669.3	79			

Table 22. Correlation of leaf length and width of *Amelanchier alnifolia/ utahensis* seedlings grown in the ecotype garden with various environmental factors defined in Table 20.

	Leaf Length	Leaf Width
Leaf width	.93	1.00
Elevation	.09	-.06
Aspect	-.13	-.12
Topographic position	.29	.53
Warm season ppt	-.59	-.42
Radiation index	.33	.35

correlation of leaf length and width is very high with strong evidence that there are large-leaved and small-leaved populations.

Elevation and slope aspect of the collection sites show no correlation with leaf length or width. Features of the environments of these populations that vary with elevation or aspect, therefore, are probably not actively selecting these ecotypes. Warm season precipitation, radiation index, and topographic position, however, are correlated with leaf size. The correlation of 0.53 between leaf width and topographic position (although not high) indicates that broader (also longer) leaves tend to be found in populations occupying lower slopes and valley bottoms rather than upland areas.

The biological meaning of this correlation is still uncertain. In fact, the negative correlations (-0.59 and -0.42) between leaf size and growing season precipitation indicate drier areas have populations with larger leaves. This is contrary to what might be expected on the basis that smaller leaf sizes in more xeric situations help to minimize water loss. Additional characteristics relevant to water loss will be measured (e.g., pubescence, stomate distribution and density) and incorporated into interpretations.

Purshia tridentata

The four populations of *Purshia* in the ecotype garden were measured for leaf length and width. Different sites were represented by six to eight individuals, and 10 leaves per plant were measured. Since the measurements were taken soon after transplanting (over the period from early July to early August) indications of genetic differences must be considered quite tentative.

Analysis of variance of leaf area index showed significant differences (.025 level) among the populations for this character. However, only one population is responsible for the difference. It has an average leaf area index of 121 mm^2 compared to 66, 72, and 72 mm^2 for the other populations.

Symphoricarpos oreophilus

This species is currently represented in the garden by 17 populations which were measured for leaf length and width, number of stems, number of leaves, and plant height. Rather than report here on variance analysis the opportunity is taken to present a different way in which we have analyzed the data. Five population variables (leaf length, width, area, shape, and plant height) were subjected to a principal component analysis. The first component extracted (Principal Component One or PC1) accounts for 72 percent of the variation among the variables and is strongly related to leaf size and plant height (Table 23). The second component (PC2) accounts for an additional 22 percent of the variation and is strongly related to leaf shape. Thus, 94 percent of the variation present in the five original variables is effectively summarized by these two newly defined variables--the first two components. Next year when more variables are added to this model, it is anticipated that the percentage variance accounted for by any single axis will go down, but the advantage of having a few variables (i.e., the principal components) summarizing many measured variables will be retained.

If plant height and leaf length are plotted against PC1, the summarizing effect can be seen (Figure 43). Leaf length and plant height correlate with one another (-0.77); however, each is more highly correlated with PC1 (0.97 and -0.87, respectively). Although not plotted,

Table 23. Correlation (loadings) of characteristics of 17 *Symphoricarpos oreophilus* populations to the first principal component (PC1).

Variable	Correlation (r) with PC1
Leaf length	.97
Leaf width	.94
Leaf area index	.98
Plant height	-.87

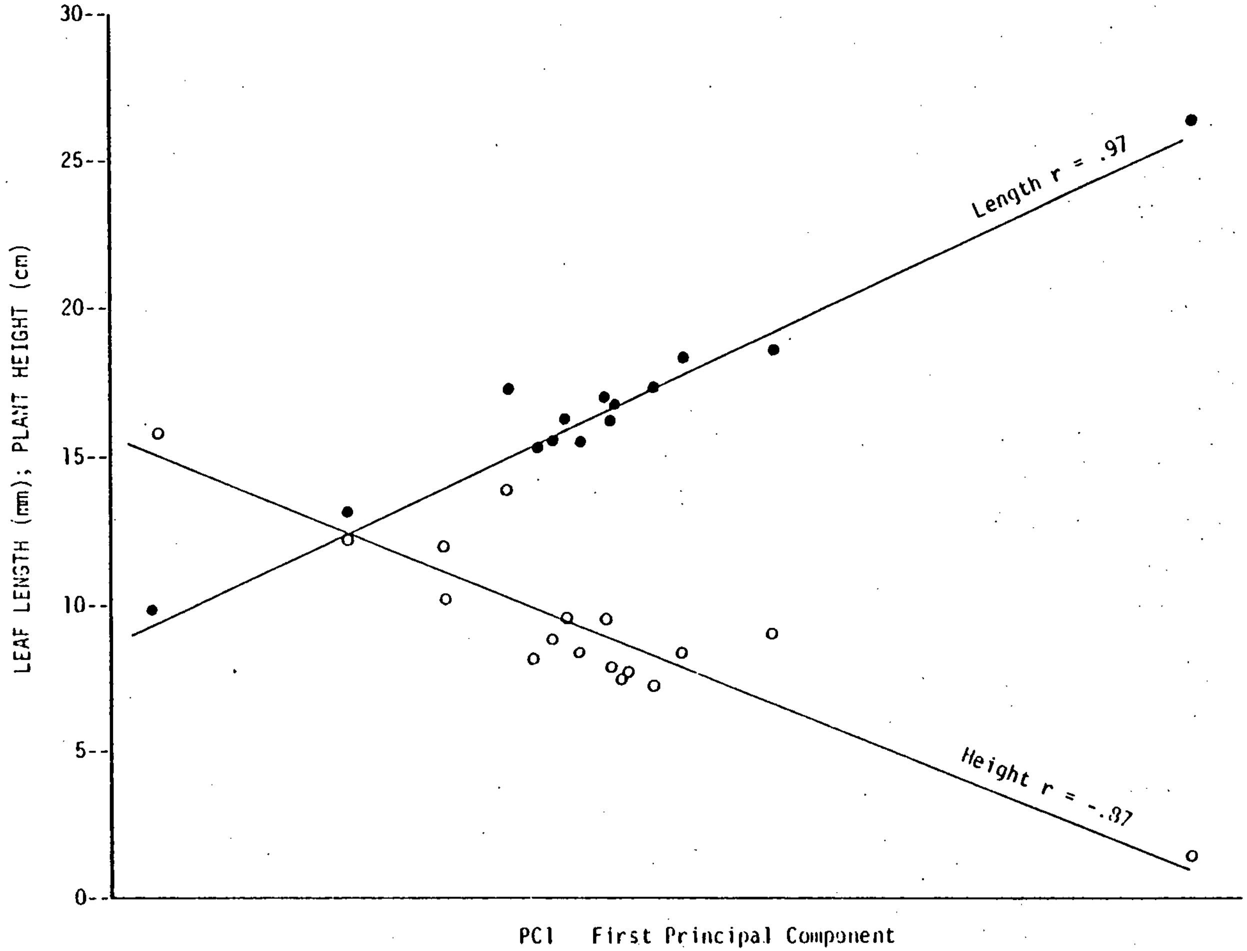

Figure 42. Relation of leaf length and plant height to the First Principal Component of 17 *Symphoricarpos oreophilus* populations.

leaf width and leaf area index are also more highly correlated with PC1 than they are to each other or to length and height.

These preliminary results suggest that the selective factors separating these populations (ecotypes) result in a trade-off between growth in height and production of phytosynthetic surface. The newly defined variable (PC1) corresponds to this hypothetical factor. Of course, this kind of analysis and these interpretations will require more adequate data. The purpose here is to show the method of analysis rather than to argue for any particular conclusion.

Populations may be plotted with respect to PC1 and PC2 (Figure 44) resulting in a simultaneous display of the relationship of plant size and leaf shape. Populations differentiated vertically (e.g., 22 and 72) will have similar leaf sizes but different leaf shapes. In contrast, Populations 19 and 55 have similar shape but different sizes.

Table 24 gives the geographic location of these 17 population sites. It can be seen that these particular ecotypic dimensions have no simple relation to geography. For example, populations plotted near one another in this ecotype space of leaf shape and plant size come from Eagle County (Site 38), the northern Front Range (Site 79), and the Piceance Basin (Sites 1, 22, 51, and 74). More complete analysis of this type will help in decisions about which populations are interchangeable as source material for reclamation.

Seedling Studies

A small experiment was conducted to evaluate growth response of seedlings of two shrub species under different environmentally controlled conditions and to learn more about experimental design for additional

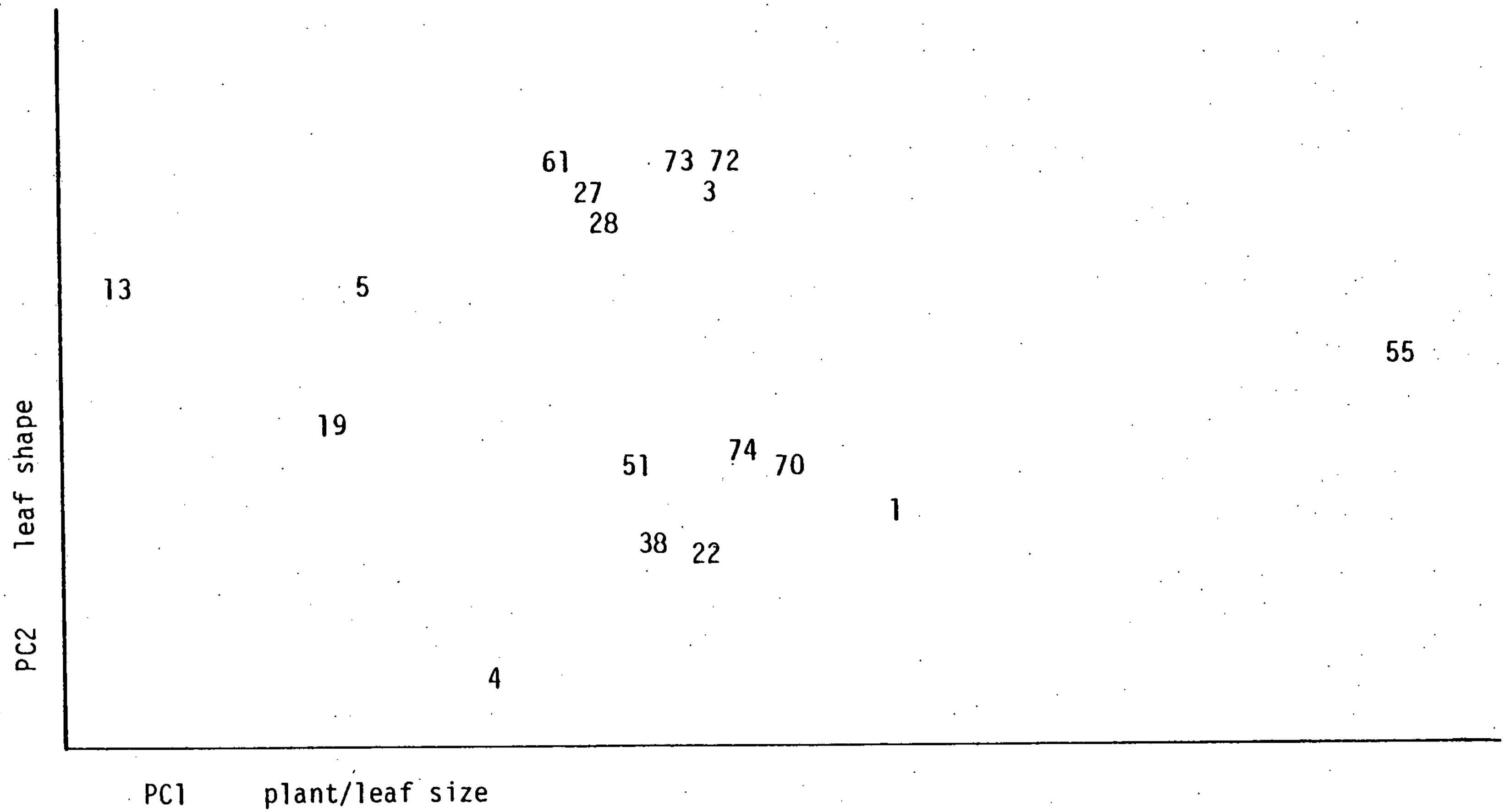

Figure 44. Location of 17 populations of *Symphoricarpos oreophilus* in the two dimensions defined by the first two Principal Components corresponding to plant and leaf size and leaf shape. (Site information for these stands is given in Table 24.

Table 24. Location of collection sites of 17 *Symphoricarpos oreophilus* populations.

Site		
1	Piceance Basin	Box Elder Gulch
3	Piceance Basin	Mouth of Cow Creek
4	Custer County, Colorado	Near Wetmore
5	Routt County, Colorado	Near Seedhouse
13	Piceance Basin	Square S Ranch
19	Piceance Basin	Dry Ryan Gulch
22	Piceance Basin	Corral Gulch
27	Piceance Basin	Tommy's Draw
28	Piceance Basin	Tommy's Draw
38	Eagle County, Colorado	Near Eagle
51	Piceance Basin	Tommy's Draw
55	Larimer County, Colorado	Poudre River
61	Piceance Basin	Colling Gulch
70	Larimer County, Colorado	Rist Canyon
72	Piceance Basin	Cow Creek
73	Piceance Basin	Cow Creek
74	Piceance Basin	Cow Creek

studies. Seeds of *Amelanchier alnifolia/utahensis* were collected at 11 sites in the Piceance Basin and one site in the Colorado Front Range. The Basin sites ranged from south-exposed, dry mesic shrub communities to cool, north-facing Douglas fir (*Pseudotsuga mensiezii*) stands. The three collection sites for *Cercocarpus montanus* included two in the Basin and one in the Front Range. The environment of each collection site was described by the 16 characteristics listed in Table 20.

The controlled environment conditions and the species populations employed in the different treatments are shown in Table 25.

After appropriate pretreatment the seeds of both species were germinated on blotters in germination boxes and then planted in commercial potting soil in 2.5x2.5x5 cm plastic pots. Individuals which attained a sturdy seedling stage of two to four leaves and a height of about 30 mm were selected for use in the experiment. Thirteen individuals represented each species population in each treatment. Individual plants were labeled by code numbers and randomly positioned in the composite of all individuals. Plants were watered as generally needed, but in equivalent amount for each treatment.

Initial measurements and notes on conditions were taken on July 10, 1978, at the time the seedlings were placed in the controlled environment chambers, and every ten days until the middle of August, 1978. Measurements were made of seedling height (soil surface to base of highest leaf), width of widest leaf, and length of longest leaf.

Amelanchier alnifolia/utahensis

Related to the combinations of temperature and light conditions, three patterns of height growth were evident:

Table 25. Species populations employed to examine seedling responses to controlled temperature and conditions.

	High Light (19,000 luxes)	Low Light (6,500 luxes)
Warm Temperatures Day --28°C ave Night--10°C	*Amelanchier alnifolia/ utahensis* Populations 1-6	*Amelanchier alnifolia/ utahensis* Populations 1-12 *Cercocarpus montanus* Populations 1-3
Cool Temperatures Day --15°C Night-- 5°C	*Amelanchier alnifolia/ utahensis* Populations 1-6	*Amelanchier alnifolia/ utahensis* Populations 1-12 *Cercocarpus montanus* Populations 1-3

1. Significant differences in height growth were recorded among populations under warm temperatures, but not under cool temperatures.
2. Most populations grew considerably more in warm versus cool temperatures, indicating plasticity of response, and
3. Neither genetically-fixed response differences nor plasticity could be related to the different light conditions.

Growth increments in *Amelanchier* under warm temperature-high light ranged from an average of 5.4 to 16.8 cm but only from 3.1 to 4.4 cm under cool temperature-high light. Under low light and warm and cool temperatures the average values for all 12 populations were 3.3 to 29.2 cm and 3.2 to 5.9 cm, respectively. Analyses of variance showed the differences among populations under warm temperatures to be significant, but not significant under cool temperatures. The cool temperature regime may be near the minimum for all populations studied, resulting in a general depression of growth and small response differences. Under warm temperatures the populations responded differently and, if placed in habitats with similar temperatures, could be expected to maintain these differences.

The relationship of response to collection site characteristics has not been fully evaluated, but a general trend of less stem growth and larger leaves with lower elevations is suggested. These same characteristics also showed some positive relationship to more shaded conditions in aspen (*Populus tremuloides*) and Douglas fir stands which, in terms of resource allocations, could be of selective advantage.

Cercocarpus montanus

For this speices the average growth increments in height and leaf width and length were, with one exception, markedly greater in the warm temperature regime. Comparing warm and cool conditions, two populations showed greater differences in leaf size while one showed more difference in stem growth.

Analysis of variance of the change in height growth in the two regimes indicated highly significant differences, and a "t-test" showed that two of the three sites were not different--but, each was significantly different from the third population. Interestingly, the different population was one of the two from the Piceance Basin and not the widely disjunct Front Range population.

Correlation of the growth chamber responses with the environments of the populations' original sites indicates that the population growing the most in height in the warm regime has an environment with a higher radiation index and less annual precipitation than that of the other populations. Conversely, those populations growing significantly more in the cool regime come from environments receiving less insolation and more moisture. This suggests the possibility of selection by factors related to radiant energy, ambient temperature, or moisture conditions. It must be recognized that the small number of populations investigated increases the chance of high correlation with such factors.

For both *Amelanchier* and *Cercocarpus* the results of the limited experiments indicate the likelihood of obtaining useful information on ecotypic differentiation and tolerance plasticity in seedlings. Temperatures between the warm and cool regimes used and somewhat above the warm would add to the picture for *Amelanchier*, with the possibility of stepwise increases in both regimes over the period of the study yielding additional benefit.

Summary of Ecotype Phase

The ecotype study has progressed to where there is the first evidence for eco-genetic differentiation among populations of *Amelanchier alnifolia/utahensis*, *Symphoricarpos oreophilus*, *Cercocarpus montanus*, and *Purshia tridentata* and where a large amount of data is soon to be available for more complete evaluations. Several methods of analysis have been tried, certain of which will substantially benefit the interpretation of the adaptive significance of this differentiation and its meaning for reclamation.

Part Two--Phytosociology

Our description and interpretation of the naturally occurring phytosociological structures in relation to their placement in the landscape serves two purposes. The first is to determine and describe the natural architecture of the areas' vegetation; this is necessary for reclamation design when the goal is the reestablishment of stable, diverse communities. The second purpose is to provide a biological context within which the interpretation of the reclamation research on the Intensive Study Site may be made. The first purpose will serve the ultimate reclamation user while the second will serve technical research interests. Both, however, are based on the ideas that the groups of species naturally found on a site are the best evidence that that kind of community will continue to be successful when restored following disturbance and that study of the distribution of species over the landscape is the only practical way to discover the factors governing community structure.

Eighty-four stands of vegetation were sampled during 1978 using a sampling regime giving frequencies of herbaceous species in 1x1.5 m quadrats, cover for shrub and tree species in three height classes on a line intercept, and density and basal area of tree species with density for three age classes in 2x10 m quadrats. Selected environmental factors and the location of each stand were also recorded. Eighteen of the 51 stands sampled in 1977 were not complete for herbaceous species due to the dry season; data for these stands were completed this year. We now have a library of 135 completed stands.

This information covers the range of vegetation variation of the predominant landscape units of the Colorado oil shale region. The distribution of stands in a broad classification of community types is given in Table 26. These stands represent a data base for developing a model of the vegetation of the Piceance Basin. For each ecotype collection site in the Basin there is a corresponding stand which will enable correlation between ecotypic and phytosociological variation. This correlation will not only aid in the interpretation of population variation but will form the basis for predictions matching ecotypes to specific sites to be reclaimed. The vegetation typical of the Intensive Study Site was also sampled in detail to provide analogue stands for research plots on the Site. The placement of the analogue stands in the phytosociological context of the region will give a basis for understanding the major directions of variation away from the Intensive Study Site--directions in which the reclamation results obtained on the Intensive Study Site will have to be projected.

Table 26. The number of stands sampled in broadly defined units of vegetation in the Piceance Basin.

Vegetation Type	Number of Stands
Desert Shrub	8
Shrub-Grass Rockland	12
Big Sagebrush-Greasewood Bottomland	10
Big Sagebrush Bottomland	5
Low Elevation Pinyon-Juniper	11
High Elevation Pinyon-Juniper	7
Pinyon-Juniper Rockland	4
Low Elevation Big Sagebrush	12
Mid Elevation Big Sagebrush	15
High Elevation Big Sagebrush	13
Mixed Mountain Shrub	20
Grassy Balds	9
Aspen Forest	4
Douglas Fir Forest	5
Total	135

Results

The stand library represents a diversity of stand types. Over 200 herbaceous species, 25 shrub species, and 5 tree species occurred within the stand set which gives (if all values taken are included) a data matrix of approximately 135 x 270. The best procedure for handling a volume of data with this high degree of diversity is to first divide it, analyze the smaller units, and then the larger divisions. This procedure minimizes the likelihood of the production of spurious and distorted dimensions of covariation of species across the stand matrix. Accordingly, analysis is now progressing through the steps necessary to classify stands into more or less homogeneous groups, ordinating each of these groups, correlating environmental parameters, determining structural and diversity patterns within each group, and finally synthesizing all of this information across groups. This lengthy procedure is using a battery of techniques from cluster analysis through reciprocal and weighted averaging, principal components analysis, and polar ordination. These results will be presented in the final report on this subproject in 1980.

In the present progress report emphasis is placed on a group of 22 stands, most of which are located in or near the Intensive Study Site or from areas with similar vegetation. Eleven stands occur within or immediately next to the Intensive Study Site, three occur about 2 km northeast, and the rest are scattered up to 25 km from the Site. The Annual Disturbance Plot and the Fertility Study Plot (established in summer 1977) were sampled before construction. Eight stands of native vegetation were chosen to serve as analogues for specific plots on the Intensive Study Site. This was done so that each study plot would have an analogue stand for inclusion in the general phytosociological analysis.

The analogue stands were chosen for their similarity to specific experimental plots based on matches of slope, exposure, and dominance aspects. The environmental measures and scalars calculated for all stands are given in Table 27.

The occurrences and co-occurrences of 42 species contained in even this small group of stands is complex enough that inspection of a raw data table of species versus stands reveals no clear patterns. Simple manipulations of the table (rearrangements of species and stands which often yield insights into the data) reveal nothing here. Accordingly, a variety of multivariate techniques especially adapted to the extraction of pattern in complex data were used here. Results from two rather different analyses are presented. First is a cluster analysis (Figure 45), a classification, of these stands based on species composition. The technique used is hierarchical and gives relative relationships of each stand to every other in terms of their overall similarity. For example, it is clear that certain stands on the Intensive Study Site are more closely related to stands outside of it than to other stands within the Site. This heterogeneity within the Intensive Study Site (note the positions of the analogues to the Successional Study Plots) will be needed in interpretations of the data obtained from the study plots. Second, to gain more understanding of the major differences in species composition among these stands a principal components analysis is presented in Table 28. Here, groups of species are discerned; each group includes species that behave alike in these stands with regard to some gradients but are responding independently with regard to other species gradients. The fact that there are several independently responding sets

Table 27. The environmental characteristics and scalars evaluated for all stands sampled.

Variable Number	Characteristics and Scalars
1	Percent Slope, measured in field
2	Aspect Scalar, NE = 1, SW = 0, = [Cos(Azimuth°-45°)+1]÷2
3	Soil Depth
4	Elevation, meters
5	Drainage Regime, 1 = >> flow off .5 = << flow off
6	Topographic Position, 1 = upland, 5 = valley bottom
7	Growing Season Potential Evapotranspiration, Apr-Oct[1]
8	Growing Season Potential Evapotranspiration, Nov-Mar[1]
9	Total Potential Evapotranspiration = Growing season + potential evapotranspiration + dormant season potential evapotranspiration[1]
10	Annual Effective Precipitation[1]
11	Dormant Season Water Deficit, Nov-Mar[1]
12	Growing Season Water Deficit, Apr-Oct[1]
13	Annual Water Deficit = Dormant season water deficit + growing season water deficit[1]
14	Equivalent solar horizontal latitude[2]
15	Radiation Index[2]
16	Soil Texture, 1 = sands, 5 = clays
17	Percent Soil Stoniness
18	Dormant Season Aspect/Slope Scalar[3]
19	Growing Season Aspect/Slope Scalar[3]
20	Dormant Season Aspect/Slope/Elevation/Topography Scalar[3]
21	Growing Season/Aspect/Slope/Elevation/Topography Scalar[3]

Table 27.--Continued

Variable Number	Characteristics and Scalars
22	Full Season Aspect/Slope/Elevation/Togpgraphy Scalar[3]
23	Index of Seasonal Differences (Variable 21-Variable 20)

[1]Estimated from water balance equations developed for Piceance Basin by Wymore (1974).

[2]Frank and Lee (1966).

[3]Low values represent most mesic sites and high values represent most xeric sites.

Locations

* Inside or very near Intensive Site
\+ Within 3 km of Intensive Site
† 8-20 km from Intensive Site

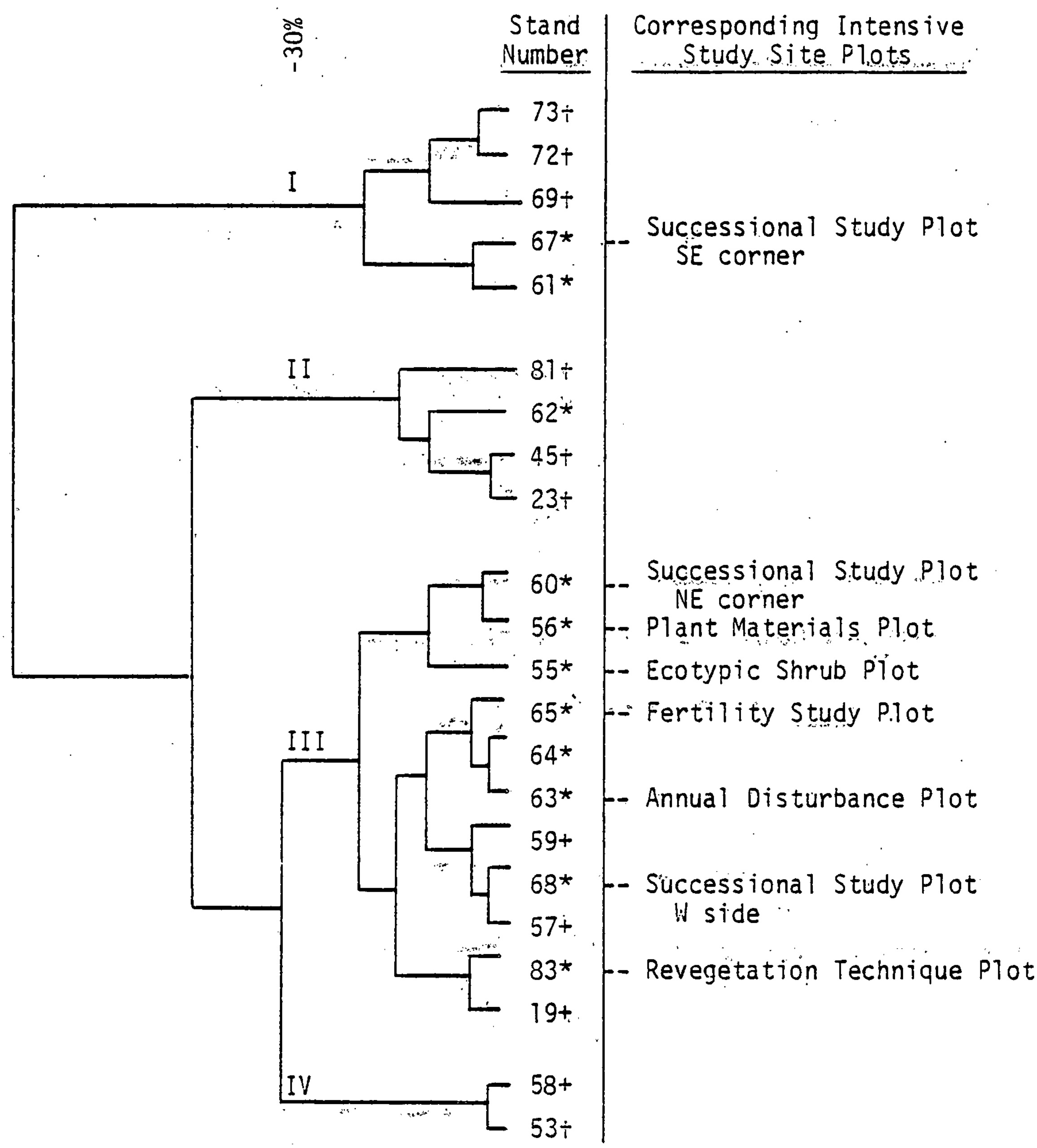

Figure 45. Hierarchical classification of stnads located in and around the Intensive Study Site. (Four groups which differ at the 30 percent relative similarity level are indicated with Roman numerals. Stands connected at higher hierarchical levels are more dissimilar.

Table 28.. Factor loadings (correlations) of shrub and understory species and environmental variables on the first three components from Principal Components Analysis of species correlations in analogue stands.

Factor Loadings	Components[1]			
	1	2	3	H[2]
Species				
Bromus tectorum	.82	.18	.02	.71
Antennaria rosea	.77	.10	.44	.79
Cryptantha flavoculata	.68	.48	.34	.80
Astragalus convallarius	.64	-.13	.43	.61
Ipomopsis congesta	.63	.64	.18	.84
Senecio multilobatus	.50	.29	.26	.41
Astragalus purshii	.45	-.38	.21	.38
Koeleria cristata	-.85	-.04	-.03	.72
Poa fendleriana	-.52	-.30	-.00	.36
Trifolium gymnocarpon	-.48	.11	.51	.49
Astragalus spatulatus	.10	.78	-.24	.67
Haplopappus nuttallii	.14	.77	-.26	.69
Phlox muscoides	-.42	.57	-.12	.51
Ceratoides lanata	-.06	.56	-.46	.52
Oryzopsis hymenoides	-.20	.56	-.41	.52
Allium nevadense	-.21	.55	.26	.40
Artemisia tridentata (low)[2]	.05	-.52	-.44	.46
Juniperus osteosperma (low)[2]	.37	-.50	-.00	.39
J. osteosperma (mid)[2]	.31	-.45	.01	.30
Artemesia tridentata (mid)[2]	.28	-.41	-.41	.41
Sitanion hystrix	.11	-.40	-.14	.36
Agropyron smithii	-.36	-.38	.50	.52
Agoseris glauca	.40	.13	.70	.67
Sphaeralcea coccinea	-.13	.19	.61	.42
Stipa comata	-.02	.27	.55	.38
Optunia (low)[2]	-.27	-.08	.55	.30
Calochortus nuttallii	-.23	-.04	.54	.34
Phlox longifolia	-.35	-.28	.54	.49
Lomatium juniperinum	-.43	.30	.51	.53
Environmental Measures				
Soil texture	-.70	-.01	.14	
Variable 20[3]	.50	-.16	.15	
Topography	-.47	.46	-.11	
Variable 18[3]	.46	.13	.39	
Potential Evapotranspiration	.46	.20	.39	
Stoniness	.20	.55	.30	
Effective Precipitation	-.15	-.52	-.07	

Table 29. Extreme values for environmental variables in the set of analogue stands and for the set of all stands. (The variables are defined in Table 27.)

Environmental Variable	Range for Analogue Stands	Range for All Stands
Elevation, m	2012-2036	1753-2621
Precipitation, cm/yr	44.1-45.1	35.1-65.7
Growing Season Potential Evapotranspiration, cm/Apr-Oct	88.1-90.8	52.3-105.8
Radiation Index	.464-.488	.224-.591
Equivalent Horizontal Solar Latitude, °N	38-43	9-67

one relevant to this subproject is that the variation within the study site may be used to show relationships of this site to other areas of the landscape. It is such information that we shall pursue during 1979.

Principal component analysis (PCA) gives a more refined view of the interrelationships of these stands since it gives a species and a stand ordination simultaneously. The PCA presented here (Table 28) was derived from the standardized frequency of 33 herbaceous and standardized cover for seven shrub species, two of which were measured in two height classes. Table 28 shows the first three components of this analysis. The first component accounts for the maximum possible variance in the data, the second accounts for the greatest amount of variance not accounted for by the first, and so on for successive components. The values (loadings) given in the table are the correlation coefficients between the species and each component. The column labeled "H^2" is the sum of the squares of the correlations of each variable on the three components given and expresses the proporation of all the variance for each species accounted for by the first three components. The proportion of all the variance in the data these components account for is .42.

Species with H^2 less than .02 were omitted from Table 28 leaving 29 species. Excluding these species here does not mean they are unimportant, only that they are responding to gradients not importantly expressed in this set of stands. For example, *Pinus edulus* and *Sarcobatus vermiculatus* occurred in a few of these stands, but this does not adequately represent their distribution across the landscape of the Piceance Basin. Further analyses including more stands will accordingly be expressive of more species patterns. In fact, the rather high proportion of species here whose variance is not related to the general pattern of variation in

Table 28.--Continued

Factor Loadings	Components[1] 1	2	3	H^2
Elevation	-.14	-.52	-.07	
Percent slope	.19	.25	.35	
Variable 8[2]	.33	-.06	.34	

[1]The highest loadings on each component are underscored. The column "H^2" gives the proportion of the variance of each variable (species or environmental measurement) accounted for by the frist three components. Total variation extracted by three axes is .42.

[2]"Low" and "mid" refer to percentage cover in height categories of less than 0.5 m and 0.5 to 1.5 m, respectively.

[3]Refer to Table 27.

of species in the data explain the obscurity of pattern in the original data matrix.

The analogue stands can be looked at from two perspectives. On the one hand, when compared with the whole range of stands in our data, they form a rather homogeneous set. For example, Table 29 gives the ranges of selected environmental factors for these stands and for the whole stand set. The elevation range is, of course, restricted because of the way in which the stands were selected. Ranges of factors reflecting moisture and energy regimes, however, are also smaller. This, too, is not surprising but points to the fact that reclamation techniques developed on the Intensive Study Site will have to be applied to areas with important environmental differences.

On the other hand, if the analogue stands are compared to one another, community and environmental variation is evident--the stands are not uniform. For example, if the stands are classified by their species compositions, four rather dissimilar groups can be discerned (Figure 45, Roman numerals). Stands chosen to correspond to particular study plots in the Intensive Site are indicated. In Figure 45 a pair of stands can be compared by counting how high up the hierarchy a path connecting them needs to go. For example, Stands 67 and 68 are connected only at the highest level which in this case corresponds to a percentage similarity separately calculated to be 69 percent. Both of these stands are located just outside the Intensive Site and were chosen because their respective slope, aspect, and other environmental variables are similar to two areas in the Successional Study Plot. Notice that each of these stands is much more similar to stands located much further away geographically (e.g., 45 and 81). Several conclusions can be drawn from such an analysis, but this stand set is the reason for the low percentage extraction by these components. As we include more stands the richness of the data set will become more apparent.

Since each component of the PCA represents an independent gradient in the vegetation, the species with especially high or low values on a component are the ones most related to it. Species with loadings near zero on a component are either not correlated to that component or are correlated with it in some nonlinear way. Species with similar loadings are responding to the gradient in a similar way while species with loadings of opposite sign are negatively correlated with each other in their response along that component; they are responding in an opposite way to some common factor.

Table 28 is arranged so that species responding to the first component are listed first, followed by those related to the second and then the third component. These species sets can be read from the table since the highest loading for each component is underscored. For example, *Bromus tectorum* and *Antennaria rosea* are highly correlated with the first component and respond to this gradient in a common way. On the other hand, *Koleria cristata* is negatively correlated with the first component; it is responding in an opposite way. Similarly, *Haplopappus nuttalli*, *Phlox muscoides*, and *Artemisia tridentata* are related to the second component. Notice that most species are correlated strongly with only one of the components, indicating that this vegetation is a mixture of at least three independent sets of species.

A helpful feature of PCA is that besides the data being factored other variables may be included in a way which does not affect the extraction of components, but does give their statistical correlation with the components. Thus, environmental variables can be included while the resulting principal component space is only determined by the vegetational variables. But still, a correlation between the environment and each component is obtained. Included in Table 28 are the correlation coefficients of selected environmental variables with each component. The values are generally low, with soil texture (r=.7 with the first component) having the highest correlation found. Investigations to see if linear or nonlinear combination of these factors, or perhaps some other factor, are more closely associated with the vegetational gradients are underway. However, since most species in the area are responding to larger portions of the environmental gradients than are represented by this limited set of stands, it is not surprising that low correlations were found. It would be dangerous to conclude at this time that the major factor to which these species are responding is a gradient of soil texture, but it is a strong possibility. As more stands are included in the analysis (that is, as a larger portion of controlling environmental factors are represented) response patterns of species to them will become evident.

The principal species components can also be used to present the stand patterns in a graphic way (Figure 46). Here the stands are arranged according to species composition. Stands with many species with strong positive correlations with the first principal component are placed high on the horizontal (or first principal stand) axis of Figure 46; stands with many species with negatively high correlations are placed low. Intermediate stands (i.e., stands with mixtures of high and low loadings) are placed inbetween. A similar procedure was carried out for the vertical axis. The analogue stands are indicated by open circles while

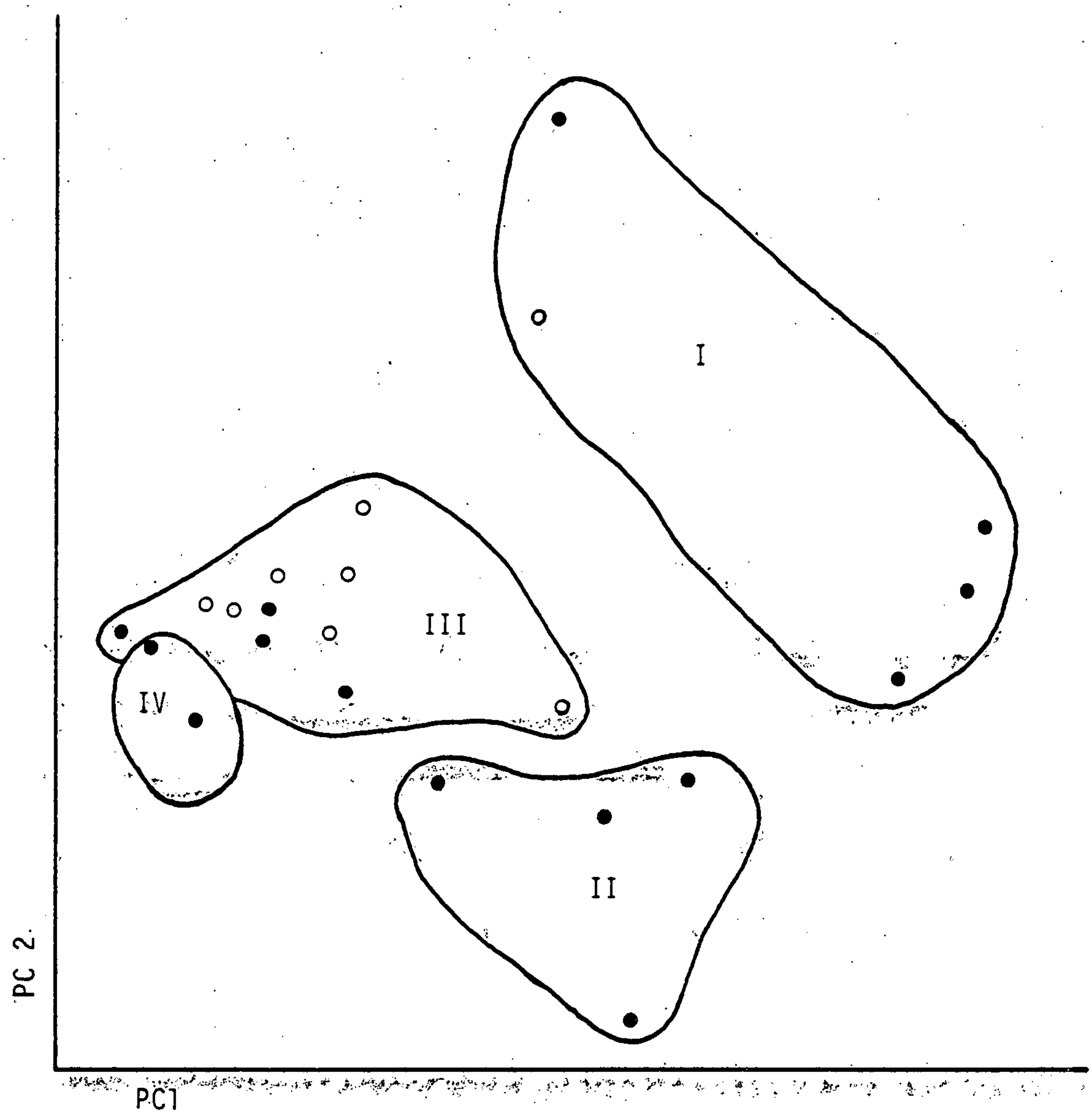

Figure 46. Display of 22 stands of vegetation in and near the Intensive Study Site components. (Stands corresponding to particular study plots within the Intensive Site are plotted with circles, the rest with points. Stands enclosed by a solid line fall within the same group as indicated in Figure 45.

other stands are represented by solid dots. The stand groups designated in Figure 45 (I through IV) were superimposed on this graph.

Further interpretation of the stand classification can now be made. For example, stands in Groups II contain proportionately more of the species negatively correlated with the second species component (e.g., *Artemisia tridentata*) and proportionately fewer of the species with high correlation with it (*Haplopappus nuttalli*). These stands also have proportionally more of those species which have correlations near zero to the first species component since these stands are near the middle of the first stand axis.

It is also possible to interpret these stand axes environmentally as the loadings (correlations) of the environmental factors on the species components in Table 28 apply to these stand axes in the same way as do the species loadings. For example, the first axis in Figure 46 corresponds from left to right to variation in soil texture from clay loam to sandy loam.

Summary of Phytosociological Phase

The phytosociological phase of this subproject has advanced to the point where virtually all field work is now complete. We have sampled 135 stands for vegetational and environmental characteristics and through preliminary tests have developed a strategy of analysis which will provide not only technical insight into the nature of the vegetational and environmental gradients present in the Piceance Basin but will also serve to structure the vegetational architecture in a way that will be immediately useful in the reclaiming of mined sites. Preliminary analyses of our data have shown the Intensive Study Site to be relatively heterogeneous in environmental and community characteristics; therefore, the interpretation of the study plots will have to be made with this in mind. This report demonstrates some of the ways in which the phytosociological data will assist this cause and how work on the Intensive Study Site may be extrapolated to the landscape of the Piceance Basin.

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ABSTRACTS OF PUBLICATIONS OR PRESENTATIONS

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ECOTYPIC DIFFERENTIATION IN SHRUBS RELATED TO GRADIENTS OF ENVIRONMENT AND VEGETATION IN THE COLORADO OIL SHALE LANDS

Six species of native shrubs, with emphasis on Amelanchier alnifolia/utahensis and Symphoricarpos oreophilus, are being studied for evidence of ecotypic variation related to gradients of environment and vegetation. Plant materials were collected mostly within the Piceance Basin of northwest Colorado, but some were obtained at distances up to 500 km from the Basin. Each collection site was characterized for integrative environmental factors (e.g., latitude, slope direction and degree, soil depth), and vegetation composition and structure were determined for collection sites in the Basin. Uniform environment conditions are provided in a common garden in the Basin, and for seedling responses, in paired growth chambers. Results show significant intraspecific differences in phenology, morphology, and growth, representing important ecotypic differentiation related to abiotic and biotic gradients. To enhance the benefits of native species in reclamation, ecotypes reasonably matched to segments of these gradients should be utilized.

This study is funded on DOE Contract No. EY-76-S-02-4018

Paper to be presented at the Annual Meeting of the
Ecological Society of America,
August, 1979

Ralph L. Dix
and
William L. Slauson

Colorado State University

THE INTEGRATION OF EXPERIMENTAL PLOT DATA INTO PHYTOSOCIOLOGICAL DISPLAY OF THE COLORADO OIL SHALE LANDS

Numerous experimental reclamation study plots were established as a part of a larger project on a single site in the oil shale lands of northwestern Colorado. The experiments include work on native and introduced species, retorted oil shale as a medium of plant growth, soil fertility, cultural practices, succession, mycorrhizal fungi and ecotypic variation. The purpose of this work is to provide guidelines for the reclamation of disturbed lands. The need to extrapolate the results from these localized experimental plots to the general plantscape has encouraged the development of an "analogue" technique where the experimental plots are matched with nearby stands of native vegetation on the basis of close environmental characteristics. This report demonstrates some of the ways by which these analogue stands and their partner experimental plots can be integrated into phytosociological displays of the regional native vegetation and into reclamation procedures.

This study was funded on DOE Contract No. EY-76-S-02-4018

Paper to be presented at the Annual Meeting of the
Ecological Society of America,
August, 1979

SELECTION OF NATIVE GRASSES AND LEGUMES FOR IMPROVED REHABILITATION

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Mr. Robert Zemetra, Graduate Research Associate

OBJECTIVES

This research involves the development of improved plant materials for the oil shale region by testing and selective breeding within a small number of important species. The hypothesis is that a few improved strains of each species could be built from genotypes of local or regional origin which would be adaptable in the rehabilitation of diverse, stable (self-sustaining), and functional ecosystems. The improvements sought are in those traits important to early growth and land protection, such as: seed size, lowered dormancy, and better seedling vigor, as well as efficient production of seed. These must be achieved without losing the ecological adaptation and the role played by these grasses and legumes in advanced successional stages.

Research on selection and breeding of plant materials is programmed along the lines of the four objectives summarized as follows:

1. Collect and assemble germplasm sources,
2. Evaluate spaced plants in two nurseries, and clonal propagules or progenies under controlled stress, for various seed and plant characters; produce test seed,

3. Evaluate seed progenies at the Intensive Study Site and satellite field plantings for survival, vigor, and adaptation, and
4. Use suitable breeding procedures for recombination of parents selected by progeny testing to develop improved strains.

A fifth objective which was included in the original (1976) objectives was to coordinate research on best means of multiplying and planting improved strains, but this objective is not at present within our means.

PROGRESS TO DATE

Research on selective breeding and testing of plant materials continued in 1978 with a less rapid pace than anticipated by the March 1978 Renewal Proposal because of a reduced budget. Work was conducted at the breeding nurseries and the trials at the Intensive Site, and the progress of research in 1978 and early 1979 is discussed under the four headings which correspond to the objectives.

Collection of Germplasm

Collecting grass seed from native sites has not usually brought the quantity needed for direct test seeding in several replications, so an attempt to shorten the time involved in nursery plant establishment was made in 1978. Pieces of western wheatgrass (*Agropyron smithii* Rydb.) were dug in the Piceance Basin in April for direct transplant to the Environmental Plant Center, Meeker. Better fertility and cultural conditions helped the ten plants from each of ten sources to yield a small quantity of seed in August 1978, but not enough for row seeding. Less difficulty was experienced with Indian ricegrass (*Oryzopsis hymenoides* (R. & S.) Ricker) which had sufficient seed in five collection sites between the Piceance Basin and Grand Junction to allow direct seedings in November at the Intensive Study Site.

Limited success was obtained with collecting lupine seed (*Lupinus argeneus* Pursh and related taxa); out of several dozen collection sites four from suitable elevation were ample enough to be seeded in the new Legume Test Plot. Most of the seed was reserved for greenhouse nitrogen fixation study and seed stock multiplication.

For the first time in three years it was possible to collect Utah sweetvetch (*Hedysarum boreale* Nutt.) at three spots in and near the Intensive Study Site, though the supply of seed was hardly sufficient to meet all our research needs.

Evaluation of the Sources in Nurseries

As stated in last year's report, spaced plant nurseries of western wheatgrass and Indian ricegrass are partially duplicated between Fort Collins and Meeker, allowing comparisoins for features of seed production and seed quality in areas which may well be involved in eventual seed multiplication of the improved varieties we are developing. A second year of seed harvest data was taken on western wheatgrass plantings in Fort Collins, and a first-year collection was made at Meeker. The first seed setting year in both locations for Indian ricegrass gave data reported hereafter, and will be followed up in 1979 harvests. Poor results were obtained with nursery growth of lupine sources at the

Fort Collins nursery, but a Meeker planting has been initiated. There is a plot of 100 plants of Utah sweetvetch (Stewart's source via Ephraim, Utah) which flowered well in Fort Collins but set only one seed pod from tens of thousands of flowers. It is suspected that this was due to scarcity or ineffectiveness of pollinators. This species also has been started in row planting in Meeker for plant-type observations and multiplication of seed of our collected strains. Data for the two grass species and the lupines are detailed under separate headings as follows.

Western Wheatgrass

In addition to the 66 plants of the elite high spread-high seed yield selections, replicated three times and maintained at the Agronomy Research Center (Fort Collins), we have 42 Piceance Basin collections approaching seed-production size. The main accession nursery of 1976 is represented by 20 plants of each of 44 sources at the Fort Collins nursery, and 15 plants each of 30 of the same sources at Meeker. Second-season seed harvest was made on the former for comparison with the results of 1977, reported last year and in Padilla's M.S. thesis (Appendix section). First year harvest at Meeker was made on individual plants in each plot, and the analysis of variance of plot means in three replications showed highly significant ($P<.01$) differneces among the sources for spread, culm number, culm height, spike length, 100-seed weight, and total seed yield per plant (Table 30). The only characters measured in the Fort Collins nursery were seed yield per plant and 100-seed weight, but both showed highly significant differences among the 45 sources (Table 31).

Table 30. Plant and seed characters of western wheatgrass at Meeker, 1978.

Source	1978 Spread Rating (0-4)	1977 Spread Rating (1-4)	Culm Number Rating (0-4)	Culm Height (cm)	Spike Length (cm)	Seed Yield (g)	100 Seed Weight (mg)
Varieties							
Arriba	3.3[1]	2.9	2.1	76.6	13.6	20.4[1]	505
Barton	3.0	2.7	2.0	70.6	13.0	22.2[1]	456
Flintlock	2.0	2.6	2.3	69.9	13.4	12.4	527
Mandan 456	2.3	2.7	3.2[1]	81.3	13.4	33.7[1]	473
Rosana	( )[2]	---	1.9	66.3	13.3	3.5	553[1]
Accessions							
A-13081	2.3	2.7	1.9	73.4	13.3	6.4	421
A-16592	3.0	2.9	1.4	62.8	11.6	4.3	382
A-16634	2.3	2.9	2.1	71.4	12.9	5.5	378
A-16931	( )[2]	---	1.2	67.4	12.7	3.4	450
BN-19823	( )[2]	---	1.3	66.2	14.1	2.2	424
BN-19824	( )[2]	---	1.6	86.8[1]	16.4[1]	8.2	517
C-27	2.0	2.6	1.7	70.2	13.4	13.3	583[1]
NM-429	( )[2]	--	1.6	80.1	15.8[1]	3.5	476
P-727	2.7	2.6	1.5	68.4	14.6	8.3	402
E6-37	1.5	2.2	0.9	58.4	13.0	3.3	444
Collections							
Colo. City	2.7	3.0[1]	3.2[1]	68.9	13.7	22.1[1]	416
Tincup	( )[2]	---	0.9	68.7	12.0	1.6	466
TS-11-11/15	2.0	2.5	1.9	69.9	12.1	6.0	426
TS-11-16/20	2.0	2.6	2.0	70.4	12.6	2.3	448
360	2.0	2.4	2.5	66.3	11.3	11.9	415

Table 30.--Continued

Source	1978 Spread Rating (0-4)	1977 Spread Rating (1-4)	Culm Number Rating (0-4)	Culm Height (cm)	Spike Length (cm)	Seed Yield (g)	100 Seed Weight (mg)
361	1.0	2.2	2.0	63.0	12.0	12.3	442
370	1.3	2.2	3.1	80.5	16.4[1]	18.6	470
372	2.3	2.3	2.4	62.5	12.0	9.4	493
378	2.3	2.8	2.3	71.0	11.0	17.8	432
385	1.3	2.1	3.5[1]	68.1	12.2	3.5	278
387	2.0	2.8	1.6	61.3	10.2	8.3	401
390	2.3	2.7	1.4	62.3	11.6	5.4	388
399	2.0	2.6	2.1	52.6	9.2	10.5	357
440	1.0	---	2.4	62.3	12.5	0.9	314
525	2.3	2.4	1.5	64.5	13.2	6.7	462
Mean (30)	2.1 ( )[2]	2.6	2.1 (24) 1.4 (6)	68.9	12.9	11.1 (24) 3.7 (6)	442
LSD (.05)	1.0 ( )[2]	0.4	1.1 (24) 0.6 (6)	12.7	2.6	10.0	99

[1]Indicates a character significantly above the mean.

[2]These six sources were planted late in 1977 so their spread, culm number, and seed yield are not comparable with the other 24 sources.

Table 31. Seed production per plant and seed weight of western wheatgrass for Fort Collins, 1978.

Source	Seed Yield (g)	100-Seed Weight (mg)
Varieties		
Arriba	17.3	498
Barton	14.8	424
Flintlock	11.7	488
Mandan 456	24.4[1]	429
Mandan 456(c)	13.8	467
Rosana	23.0[1]	510
Rosana(c)	32.6[1]	548[1]
Accessions		
A-13081	9.0	513
A-16592	11.7	577[1]
A-16634	6.2	405
A-16931	11.7	396
C-27	6.8	483
C-30(c)	13.8	538
NM-429	15.6	488
P-727	11.2	449
E6-37	3.4	371
Collections		
Colo. City	16.3	462
Tincup	16.2	446
TS-11-11/15	3.6	498
TS-11-11/15(c)	3.4	516
TS-11-16/20	3.9	412
001(c)	23.5[1]	532
011(c)	12.0	365
081(c)	12.3	455
171(c)	6.0	385
271(c)	5.1	322
292	6.3	397
319	5.3	399
357	8.1	464
360	11.8	435
361	7.2	469
370	8.7	495
372	6.8	418
373	9.2	385
378	14.6	447
385	3.3	391
387	6.8	449

Table 31.--Continued

Source	Seed Yield (g)	100-Seed Weight (mg)
390	10.0	399
399	1.4	444
411(c)	11.7	428
440	0.2	328
521	3.6	441
525	7.8	500
232120	5.7	386
232121	6.1	463
Mean (45)	10.3	447
LSD (.05)	8.0	100

[1]Indicates a character significantly above the mean.

In both nurseries the named varieties have most of the high seed yields and tend to show higher than average 100-seed weights. This might be expected from their past history of selection and makes them good candidates for further selection since there is still much variability from plant to plant. Despite such variability, the source averages are found to differ significantly (Table 32). The nonsignificant correlations for spread, seed yield, and 100-seed weight between Meeker 1978 and Fort Collins 1977 are probably due to genotype-environment interaction with both season and location being different. The other correlations showed a surprisingly consistent behavior; for instance, the highly significant r=0.70 between 1978 and 1977 of 184 single plants meausured for 100-seed weight at Fort Collins suggests that seed weight is an intrinsic trait.

The intercharacter correlations in western wheatgrass showed, as in 1977, that seed yield is positively correlated (+0.40) with spread and (+0.49) with culm number. On the other hand, seed yield was not significantly related (r=0.27) to 100-seed weight at Meeker; whereas at Fort Collins (r=0.47), it was highly related. Since both characters are desired when selecting superior plants, the positive correlation is helpful.

Selection of the best individuals within varieties, accessions, and collections in this project is based upon a combination of spread, seed yield over 30 g per plant (there were 63 of these in the Fort Collins 1978 harvest), and 100-seed weight over 500 mg. It can be seen from Table 33 that there is a range of values available for selection, and the opportunity exists for genetic improvement with these traits. In order to test the value of heavy seeds in establishment, as well as

Table 32. Correlations of western wheatgrass characters.

	Nurseries and Years	r	n
<u>Character</u>			
Spread	M-78 to M-77	0.78	23
	M-78 to M-77	0.30	23
Seed yield	M-78 to F-77	0.37	27
	M-78 to F-78	0.58[1]	28
	F-78 to F-77	0.49[1]	44
100-Seed weight	M-78 to F-77	0.36	27
	M-78 to F-78	0.43[2]	28
	F-78 to F-77	0.70[1]	184[3]
<u>Intercharacter correlations at Meeker in 1978</u>			
Seed yield and 100-seed weight		0.27	30
Seed yield and culm number		0.49[2]	30
culm height		0.39[2]	30
spike length		0.11	30
spread		0.40[2]	24
100-seed weight and culm number		-0.21	30
culm height		0.42[2]	30
		0.43[2]	30
		0.20	24
<u>Intercharacter correlations at Fort Collins in 1978</u>			
Seed yield and 100-seed weight		0.47[1]	45

[1]Significant at P<.01.

[2]Significant at P<.05.

[3]Correlation in 184 single plants.

Table 33. Extreme values and selected plants for progeny testing from the western wheatgrass nurseries.

Character	Lowest	Highest	Selected Individuals mean	(range)
Meeker				
Spread (0-4)	1	4	Scored on plot basis	
Culm number (0-4)	0	4	2.8	(2-4)
Culm height (cm)	30	107	No selection pressure	
Spike length (cm)	5	25	No selection pressure	
Seed yield (g)	0	80.5	24.4	(2.6-80.5)
100-seed weight (mg)	251	752	567	(501-752)
Fort Collins				
Seed yield (g)	0	82.4	40.7	(30-82)
100-seed weight (mg)	208	685	554	(501-685)

adaptation, progenies of the highest 100-seed weight plants were seeded at the Intensive Study Site in November 1978, and more will be included next season.

Indian Ricegrass

The nurseries established at the Agronomy Research Center and the Meeker Environmental Plant Center in 1977 each contained 16 sources. The total of almost 480 plants was harvested for seed at the two locations in 1978, except for a few plants that were infected with smut and aborted their seed panicles. Some additions to the two nurseries, including collections made by Dr. Lang (University of Wyoming) and a minor representation of collections of *Oryzopsis* made by Dr. Ward (CSU) were not large enough to yield harvestable seed or reliable data in 1978 but will be followed in 1979, as will the individual plants of the two 16-source nurseries for their second harvest year.

The first-year comparisons (Table 34) for plant shape, panicle number, panicle height, relative maturity, seed yield per plant, and 100-seed weight in Indian ricegrass were analyzed statistically with variation in some of the measures being highly significant among sources. Plant shape, panicle number, flowering date, and 100-seed weight source differences were all significant at Fort Collins, thus showing that strains could be distinguised for those characters. At Meeker only plant shape and 100-seed weight were significantly different among selections, while panicle height and seed yield were nonsignificant in both nurseries. Among the 12 or 13 entries which are actually duplicated between the two nurseries, there are significant nursery-to-nursery correlations for plant shape, panicle number, seed yield, and 100-seed wieght (Table 35).

Table 34. Plant and seed characters of Indian ricegrass at the Fort Collins and Meeker nurseries, 1978.

Source	Plant Shape[1] (1-4)		Panicle Number[2] (1-4)		Panicle Height[3] (cm)	Panicle Height[3] (1-3)	Flowering Date[4] (days)	Maturity[5] (1-3)	Seed Yield per Plant (g)		100-Seed Weight (mg)	
	F	M	F	M	F	M	F	M	F	M	F	M
<u>Varieties</u>												
Breeders	3.3	4.0	3.3	3.0	46	2.7	6.0	1.0	9.9	3.1	345	331
Paloma	1.3	1.5	4.0	3.7	54	2.0	5.7	2.0	11.4	4.3	444	436
Sharps	1.3	1.0	4.0	3.5	49	2.0	7.7	2.0	11.5	4.2	460	408
Warner	3.0	---	3.3	---	39	---	4.7	---	6.1	---	357	---
M-700	1.7	2.5	4.0	3.5	37	1.0	8.5	1.5	12.6	2.9	272	241
<u>Accessions</u>												
NM-15	1.3	2.5	2.7	3.3	36	2.0	8.0	2.0	4.2	2.1	385	409
NM-168	1.0	1.0	4.0	4.0	44	2.0	6.3	2.0	16.7	4.9	469	422
P-2575	3.5	4.0	3.5	3.0	54	3.0	6.5	1.0	11.3	3.2	335	336
P-15597	3.0	3.7	3.7	3.0	46	2.3	5.0	1.0	9.6	3.4	323	307
P-15598	2.7	2.5	3.7	4.0	39	2.0	8.0	1.5	10.5	1.9	338	298
P-15650	3.7	4.0	3.3	3.0	41	2.0	4.0	1.3	4.6	2.6	271	275
P-15657	1.7	1.5	4.0	3.0	47	1.3	4.3	2.0	5.9	1.6	290	318
16503-65	---	1.8	---	3.5	--	1.5	---	2.0	---	2.9	---	399
525-36	---	4.0	---	3.0	--	2.0	---	1.0	---	3.1	---	275
Collections												
E-1	2.7	---	2.3	---	46	---	3.3	---	2.4	---	286	---
PB-1	---	4.0	---	3.0	--	2.0	---	1.0	---	2.3	---	244
PB-2	1.7	2.0	2.0	2.3	36	2.0	4.3	1.0	2.2	1.0	287	341

Table 34.--Continued

Source	Plant Shape[1] (1-4)		Panicle Number[2] (1-4)		Panicle Height[2] (cm)	(1-3)	Flowering Date[4] (days)	Maturity[5] (1-3)	Seed Yield per Plant (g)		100-Seed Weight (mg)	
	F	M	F	M	F	M	F	M	F	M	F	M
PB-4	3.3	2.5	2.3	2.0	47	2.0	4.7	1.0	1.2	[6]	178	[6]
SC-1	2.0	---	2.5	---	41	---	7.0	---	2.0	---	304	---
Mean	2.3	2.6	3.3	3.2	42	2.0	5.8	1.5	7.6	2.9	336	340
LSD (.05)	1.0	1.0	1.0	ns	ns	ns	2.5	ns	ns	ns	72	60

[1]1 condensed to 4 open, at F = Fort Collins, M = Meeker

[2]1 with 0-5 panicles to 4 with over 30 panicles per plant.

[3]Height at Meeker, 1 short to 3 tall.

[4]Days from June 9, 1978.

[5]1 was mature before July 18, 2 mature on July 18, 3 not mature.

[6]No seed data available for PB-4 at Meeker.

Table 35. Correlations between two nurseries for various plant and seed characters in Indian ricegrass and their intercharacter correlation.

	r	Number of Entries
100-seed weight M to FC	+0.90[1]	12
Seed yield M to FC	+0.81[1]	12
Plant shape M to FC	+0.87[1]	13
Panicle number M to FC	+0.75[1]	13
Panicle height M to FC	+0.44	13
Flowering date (FC) to maturity (M)	+0.38	13

Intercharacter correlations: Meeker above diagonal, Fort Collins below diagonal. Meeker: (15-16 entries)

Fort Collins (16 entries)	100-Seed Weight	Seed Yield	Plant Shape	Panicle Number	Panicle Height	Maturity
100-seed weight	x	+0.47	-0.67[1]	+0.38	+0.14	+0.68[1]
Seed yield	+0.66[1]	x	-0.21	+0.55[2]	+0.20	+0.30
Plant shape	-0.56[2]	-0.27	x			
Panicle number	+0.53[2]	+0.83[1]		x		
Panicle height	+0.23	+0.31			x	
Flowering date	+0.39	+0.50[2]				x

[1]Significant at $P<.01$.

[2]Significant at $P<.05$.

These correlations suggest that there are varietal differences in these traits which remain characteristic in different places. Thus, Paloma and NM-168 strains of Indian ricegrass are condensed, compact plants with many panicles, high seed yield, and heavy seeds in contrast to P-15650 and the Piceance Basin collections which are more open in growth, have fewer panicles, lower seed yields, and small, light seeds.

Correlations between one trait and another show some expected relationships, such as, seed yield to panicle number. An interesting strong, negative correlation is that between plant shape and 100-seed weight with the "condensed" plant shape having the heavier seeds. Neither panicle height nor flowering date have much relationship to seed characters except that in Meeker the later-maturing Indian ricegrass types tend to have heavier seed. It is hoped next season to harvest seed at their own natural maturity over a more extended period since these plants are somewhat indeterminate in their seed setting.

Single-plant selections of Indian ricegrass have been made for progeny testing based on high seed yield and heavy 100-seed weight in the belief that both characters are important for the most efficient rehabilitation technology. Such selection criteria was suggested by the results of varietal source tests in the Piceance Basin environment where the progeny tests are also under way. There is a wide latitude of selection possible as individual plants ranged from 0 to 31 g of seed per plant and from 107 to 617 mg per 100 seeds. These may be contrasted with entry means up to 16.7 g of seed and weights up to 469 mg. In both traits selection NM-168 was the leading variety, and it also had the plant with the highest extremes. An intervarietal recombination of the five sources

Paloma, Sharp's, Warner, M-700, and NM-168 has been planted while waiting for the results from the single-plant selection program.

One character of great importance in Indian ricegrass is seed dormancy. As indicated in the 1977 report, dormancy-breaking treatments (both mechanical and chemical) were studied by comparing their effects in laboratory and field germination so as to seek one which would be effective in critical-site testing of the fresh seed of progenies and field collections.

In the laboratory, tests were done on ten treatments applied to two varieties and three ages of seed. The percentage mortality was obtained from a tetrazolium test on all seeds which had not germinated after four weeks. There were no significant differences in mortality (Table 36) for the variety Warner in any of the three ages of seed. The higher percentage mortality in the variety Paloma, especially in 1977 seed, was most likely due to the method of cleaning (processing) the seed after its harvest and was not related to the scarification treatments. This demonstrates the need for proper cleaning of seed for revegetation work. At the same time, the results on both varieties showed that the scarification treatments did not cause excessive damage to the Indian ricegrass seeds.

In the field, a test of the scarified seed was planted at the Intensive Study Site in October 1977. Only the strain Warner was planted due to the unavailability of Paloma seed at the time of planting. From the data collected (Table 37), the acid scarification (the best in the greenhouse study as reported in 1977) was shown to be detrimental to the germination of Indian ricegrass in the field. This was most likely due

Table 36. Percentage mortality[1] of Indian ricegrass seed tested in the laboratory after scarification treatments of different-aged seed.

Treatment	Warner			Paloma		
	1975	1976	1977	1975	1976	1977
Control	6.3	4.0	9.0	16.7	32.3	48.0
Commercial scarifier	5.7	2.7	9.0	12.7	29.7	45.7
Tumbler	6.3	3.0	8.3	21.0	24.3	37.0
Rubbing	3.3	5.7	8.3	17.7	22.7	32.7
Acid	2.7	8.3	10.0	16.0	24.0	45.0
Control and GA_3	11.0	13.3	14.5	21.7	23.0	49.7
Commercial scarifier and GA_3	8.3	5.7	13.0	21.7	28.0	39.3
Tumbler and GA_3	8.0	8.3	9.7	13.0	16.7	39.7
Rubbing and GA_3	8.7	8.0	9.7	14.0	25.3	45.3
Acid and GA_3	6.3	3.7	10.3	8.3	23.7	31.0
	LSD(.05) = 10.8			LSD(.05) = 11.4		

[1]Total viability (germinated + live) = 100 - mortality. The greenhouse germination percentages were reported in the Progress Report, March 1978 (Table 36, p. 134).

Table 37. Germination percentage in the field (Intensive Study Site) for scarified Indian ricegrass seed of different ages.

Treatment	Warner		
	1975	1976	1977
Control	15.0	13.5	8.2
Commercial scarifier	12.5	17.8	10.2
Tumbler	10.0	15.5	8.5
Rubbing	23.7	9.8	12.0
Acid	6.0	2.8	3.0
Control and GA_3	18.0	11.0	8.6
Commercial scarifier and GA_3	15.5	16.5	12.8
Tumbler and GA_3	15.5	14.0	8.8
Rubbing and GA_3	20.0	17.2	5.8
Acid and GA_3	4.5	2.5	3.8
		LSD (.05) 4.6	

to the acid treatment weakening the seed coat so much that viability could not be maintained by the seed over winter in the soil.

The mechanical treatments in some cases improved the germination slightly and in others significantly, indicating that with modification of the mechanical scarification methods germination can be improved. At the moment no one method could be recommended as a treatment.

Lupines

The nursery planting in Fort Collins did not yield usable seed in 1978, and it is evident that there are also problems with collecting seed from native stands. So, the limited seed thus gathered in 1978 has been devoted to two types of research. One is the legume adaptation study at the Intensive Study Site which will meausre nitrogen fixation in the environment of the oil shale area. The other is a screening study designed to identify differences among strains (sources) in their capacity to fix nitrogen in a controlled greenhouse environment.

A preliminary seeding of ten strains in October 1977 at the Intensive Site resulted in good stands of seedlings visible on April 7, 1978, but these were eaten by rodents below the cotyledons some time before April 28. This type of grazing damage is not compatible with strain-testing with limited quantities of seeds. The raising of plants for seed-setting at the Intensive Study Site is again being attempted by the October 1978 seeding in the fenced area of the Legume/Nitrogen Fixation Plots. Another approach cooperative with EPC at Meeker has been to seed one row each of about 20 collections in their better-managed seed-farm environment. These rows are intended for plant type evaluation and production of seed for seeding trials in the Piceance Basin.

Testing Source Material and Progenies in the Critical Environment

The testing of material in the critical environment is the most essential step in proving which commercial varieties, strains, accessions, ecotypes, or single-plant progenies can succeed in the rehabilitation process. Moreover, by identifying the best mother plants on this criterion the breeder can put together the synthetic strains which are needed for that specific task. Some strains are synthesized for salt tolerance, low dormancy, or nitrogen-fixation efficiency; but, all have to have adaptation to establishment, survival, and growth in the Piceance Basin.

One test which was planted in 1976 at the Intensive Study Site was a split-plot test in which the different grasses and legumes could be compared among strains within species; they will be so discussed under separate headings. The other similar strain test planted in 1976 at a mountain browse site in the Piceance Basin was examined in June 1978 with the conclusion that poor initial performance of many entries through the dry winter and summer of 1977, coupled with the luxuriant growth of native invaders, dictate its abandonment. The progeny tests planted at the Intensive Study Site to test selections of western wheatgrass and Indian ricegrass from elite mother plants are described below in separate sections.

Western Wheatgrass

For the 1976 strain test the ratings of survival on May 22, 1978, and growth on September 23, 1978, both showed highly significant differences in favor of Arriba, Mandan 456, Rosana, and C-30 (Table 38).

Table 38. Second growing season performance (1978) compared with 1977 at the Intensive Study Site for western wheatgrass (seeded 1976).

Accession	Vigor 9-02-77	Survival 9-22-78	Growth 9-23-78	Spread 9-78	Seed 9-78
Arriba	3.4	3.5[1]	3.5[1]	S[2]	XX[3]
Barton	1.7	1.5	1.5	S	X[4]
Flintlock	1.9	1.5	2.5[1]	S	X
Mandan 456	2.9	2.5[1]	3.0[1]	S	X
Mandan 456 (OP)	2.5	2.5[1]	3.0[1]	SS[5]	X
Rosana	1.1	0.5	1.0		
Rosana (OP)	3.0	3.0[1]	3.0[1]	S	X
A-13081	0.4	0.0	0.5		
A-16592	0.0	0.0	0.0		
A-16634	1.0	0.0	0.0		
A-16931	1.3	1.5	2.0	S	X
C-27	0.0	0.0	0.0		
C-30 (OP)	3.5	4.0[1]	4.0[1]	S	X
NM-429	0.6	0.5	0.5		
P727	1.7	1.5	2.0		
Tincup (OP)	1.9	1.5	2.5[1]	S	X
C-26	0.0	0.0	0.0		
C-178	0.0	0.5	0.0		
C-212	0.0	0.0	1.0		
NM-51	0.0	0.0	0.0		
NM-95	0.0	0.0	0.5		
NM-290	0.0	0.0	0.0		
NM-293	0.0	0.5	0.0		
NM-381	0.0	0.5	0.5		
Victor	0.0	1.5	1.0		
Mean of test	1.1	1.1	1.3		
LSD (.05)	---	1.1	0.8		

[1]Significantly above the mean of the test, on 0-4 rating.

[2]S = Spreading by rhizomes.

[3]XX = Setting seed in many spikes.

[4]X = Setting seed in spikes.

[5]SS = Spreading strongly.

New seed accessions planted in April 1978 did not perform well owing to the dry mid summer. This confirms the need for using seed of known dependable quality produced in nurseries or seed fields, as well as using the optimum fall planting date and proper soil preparation.

The replicated progeny test of 108 single-plant progenies of western wheatgrass (sown in early April) showed some emergence and growth by September, although disapointingly small. Nevertheless, there were highly significant ($P < .01$) differences among progenies, with a mean rating of September growth of 0.87 on a 0-4 scale. The top 31 entries were classified as relatively vigorous (means 1.25 to 2.25), and their proportional occurrance in the groups of parental material (Table 39) can be seen to support the hypothesis that selection within varieties previously chosen as good seed producers is more likely to result in vigorous progenies. Selection of parents based on a combination of seed production, seed size, spread, and proven progeny performance in the test site appears to identify superior strains.

Another progeny test planted at the Intensive Study Site in November 1978 includes some of the same entries identified earlier and also 23 newly identified single-plant progenies from the Meeker nursery. Selection of the latter was based on their having 501 to 752 mg 100-seed weight, averaging 567 mg, compared to the Meeker nursery average of 442 mg.

The survival and growth of all the 1978-planted progeny tests has to be followed into 1979 and 1980 to be sure that we are identifying superior plants for the reclamation process.

Table 39. Progeny tests of western wheatgrass at the Intensive Study Site.

Source of Plants in the Spaced-Plant Nursery	Parents Selected (November 1977)	Progenies Good in Vigor (September 1978)	
	Number	Number	Percent
Named varieties: Arriba, Barton, Flintlock, Mandan 456, Rosana	29	13	45
SCS--Plant Materials accessions	15	5	33
Collections: CO, MT, UT, WY	15	6	40
Collections: Canada	49	7	14
TOTAL	108	31	
Total plants in source nurseries: 1,735			

Indian Ricegrass

The 1978 ratings of the 1976 strain test show continued superiority of the varieties Sharp's, Warner, M-700, and the Soil Conservation Service strain (NM-168) along with three or four others reaching the 3.0 rating (Table 40). Noteworthy are the continued low scores of local collections, except perhaps PB-4 from the Piceance Basin. The collections of 1976 seed expressed high dormancy revealed by substantial numbers of seedlings emerging in spring 1978. This was up to five times the number which emerged in 1977.

A second accession nursery incorporating some of Dr. Lang's collection (from Wyoming) and some of our own revealed June emergence from April 1978 planting to be good for two out of ten entries--Lang 8 and J-5, the latter from Gardner, Colorado (northwest of Walsenburg). Unfortunately, heavy rodent or other predation resulted in these rows being heavily damaged.

The first test of spaced-plant progeny used the 30 best selections identified in 1978 from the Fort Collins and Meeker nursery plants. As dormancy testing data are gathered, the intention is to add these to the testing program of elites. We will also add the seed shed at maturity on the ecotype study garden plants of *Oryzopsis* to the 1979 fall test-seeding for proper comparison.

Lupines, Utah Sweetvetch, and Other Legumes for Nitrogen Fixation

From the first this subproject has included legumes of native and non-native species in the evaluation plots. Those in the 1976 strain tests did not show up as well in 1978 as in 1977. Because of rodent damage in 1978, it was decided to join forces with the Fertility

Table 40. Second growing year performance (1978) on Indian ricegrass sources planted November 1976 at the Intensive Study Site as compared with 1977.

Source	Year Collected	Dormancy[1] (%)	Vigor 9-77 (0-4)	Survival 5-78 (0-4)	Growth 9-78 (0-4)
Varieties					
Breeders	1972	3	2.5	4.0	3.0
Paloma	1974	6	2.3	3.5	3.0
Sharp's	1974	28	2.8	3.5	3.5[2]
Warner	1975	62	4.0	4.0	3.5[2]
M-700	1972	17	2.9	4.0	3.5[2]
Accessions					
NM-15	1965	5	1.9	2.0	2.0
NM-168	1968	3	3.6	4.0	4.0[2]
P-2575	1971	14	2.6	3.0	1.5
P-15597	1969	4	3.0	3.0	2.5
P-15598	1965	2	3.0	4.0	3.0
P-15650	1971	20	2.4	3.0	3.0
P-15657	1972	20	3.1	3.5	2.0
16503-65	---	2	0.8	1.0	1.5
Collections					
E-1	1976	51	1.0	1.5[3]	1.5
R-1	1976	48	0.3	2.0[3]	1.0
PB-1	1976	44	0.5	1.5[3]	1.5
PB-2	1976	28	0.5	1.0[3]	1.0
PB-4	1976	44	1.8	2.5[3]	1.5
Mean			2.2	2.8	2.4
LSD (.05)			---	1.6	1.0

[1]Dormancy in March 1977; see 1977-78 report.

[2]Significantly above the mean of the test, on a 0-4 rating.

[3]New seedlings emerging.

Subproject and test major and minor strains of legumes on a new test site designed to answer questions about the effectiveness of nodulation, nitrogen fixation, and nitrogen supply to the soil and the associated grasses. Details on plot construction and experimental design are discussed earlier in this report by Drs. Berg and Sabey. The species used in this study are listed in Table 41. The minor strains are replicated two or three times depending on quantity of seed available for the two 2 m rows in each replication. A total of 180 rows was planted. Observations in the fenced plot area in spring 1979 will be followed by more detailed studies of growth, flowering, seed setting, and also of nodulation and root-system acetylene reduction as indicators of nitrogen fixation.

Preliminary indication from another survey have shown more inorganic N in the soil analyzed from around lupine plants than in soil from adjacent areas without lupines, suggesting that these legumes are contributing N to their environment. More quantitative estimates are to be sought from those places as well as in the better-defined system of the plots in the Intensive Study Site.

Recombination of Breeding Material

Three recombination blocks have so far been established to get interpollination of the identified superior germplasm. The 66 elite clones of the first western wheatgrass nursery, all of which are in the Intensive Site progeny test, are in a three-replication, 198-plant recombination block scheduled for major seed production in summer 1979. The less desirable plants in it can be cut back before flowering to

Table 41. Legumes seeded for adaptation and N_2-fixation study.

Species	Major Plot (broadcast)	Minor Strains (in rows)
Utah sweetvetch	Stewart's[1]	Stewart's, ISS, E/ISS, Pipeline
Alfalfa	Ladak	Ladak
Cicer milkvetch	Lutana	Lutana, C-4, Dotzenko, Strohman, Sugarbeet-2, Wellington, 20-15
Crownvetch	Penngift	Penngift, Emerald, Chemung
Sainfoin	Eski	Eski, Melrose, Remont
Lupine	---	031, 143, 255, 232
Purple prairieclover	---	Kaneb
Swainson's pea	---	San Luis Valley

[1]With and without local soil for rhizobial inoculum. All others are preinoculated with the most appropriate inoculum.

remove them from contributing pollen. Other blocks scheduled in early spring 1979 (this contract year) are for elite plants identified in Meeker and for the 30 heaviest-seeded western wheatgrass plants.

Indian ricegrass breeding has advanced to the making of one intervarietal recombination with 20 plants each of Paloma, Sharp's Warner, M-700, and NM-168. A low-dormancy block was planted from seedlings which emerged rapidly (i.e., were nondormant) in the controls of the scarification study. These were able to set seed in September 1978 at Fort Collins under supplemental irrigation, and this first seed crop is undergoing dormancy testing. The main seed crop is expected in July 1979 and will be compared with the first-year crop whose environment during seed maturation was not quite normal for the species. Other recombinations will be set up as elite plants for various traits.

Summary

Several strain tests are underway at the Intensive Study Site. Western wheatgrass, Indian ricegrass, and several leguminous species have been evaluated during the 1978 growing season. Results indicate that varieties Arriba (C-30), Mandan 456, and Rosana are the best performers for western wheatgrass and provide (along with other sources) individual plants with traits superior to the variety averages. Seed size, seed yield, spread, and survival are all important characteristics being closely monitored.

Among the varieties and accessions tested for Indian ricegrass, it appears that Sharp's, Warner, M-700, and SCS's strain (NM-168) provide the highest vigor, growth, and survival ratings. Comparisons to local collections have been difficult because of high dormancy levels in this seed.

Major and minor strains of lupines, Utah sweetvetch, cicer milkvetch, and other legumes have been planted for testing in 1979 for their effectiveness to fix nitrogen in native soil at the Intensive Study Site. This work is being carried out in conjunction with fellow scientists from the Departments of Agronomy and Microbiology at Colorado State University.

ABSTRACTS OF PUBLICATIONS OR PRESENTATIONS

ABSTRACT OF THESIS

EVALUATING WESTERN WHEATGRASS SOURCES FOR
OIL SHALE LAND REVEGETATION

Western wheatgrass (*Agropyron smithii*, Rydb.) will be among those species used for rehabilitating the disturbed land from oil shale development in northwestern Colorado. A variety of this species specifically adapted for the Piceance Basin is not available. To insure successful reseeding and ecosystem stabilization, such a western wheatgrass variety is needed. Evaluation of different seed sources is the main purpose of this study. Relationships between characters used to improve screening are also studied, as are geographical pattern which may exist for these characters.

From space-planted nurseries in Fort Collins and Meeker, Colorado, wide genetic variability necessary for successful plant breeding was found among 44 sources constituting varieties, PMC numbered accessions, and collections. Highly significant differences were present for all four of the vegetative and six of the seven seed related characters. Results discussed are for only one particular year, which proved to be a very dry one, affecting results greatly; growth at Meeker was much less than at Fort Collins. Correlations between nurseries for spread and culm number ratings were low, therefore selection of types adapted to the climate of northwestern Colorado can be made only indirectly at Fort Collins, where much of the data is available.

In seeding trials in the Piceance Basin, Arriba and open-pollinated progenies of Rosana and C-30 were the better sources

after one season's data on emergence and seedling establishment. Selection of individuals for progeny testing in the Piceance Basin will include superior plants from the above three sources plus others with specific desirable characteristics.

Five correlations between nursery characters proved to be meaningful (r^2 greater than or equal to .5). Of these five, the interrelatedness of three was most important. They were herbage and culm number (r^2=.51), herbage and seed yield (.56), and culm number and seed yield (.58). Seed yield, whose direct evaluation is laborious, can be reasonably predicted by herbage and/or culm number.

Only vegetative height displayed any meaningful correlation with geographical origin (latitude and elevation). The effect of lattitude was most responsible for vegetative height, (r^2=.56), when the effect of elevation was controlled. Other environmental factors may be responsible for the variation in the other characters.

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April, 1978

-- ABSTRACT FORM -- 1978 AGRONOMY ABSTRACT --

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FORM B -- Complete one (1) copy. Follow Directions below. Indicate Division(s) ____________________

TYPE ABSTRACT HERE

Reducing Seed Dormancy in Indian Ricegrass for Disturbed Land Reclamation, R. S. Zemetra and R. L. Cuany

Less seed dormancy in Indian ricegrass, *Oryzopsis hymenoides* (R.&S.) Ricker, would increase its usefulness in reclamation. Two approaches have been initiated, seed scarification and breeding a reduced dormancy strain. Three years, (1977, 1976 and 1975), of Indian ricegrass seed were scarified by three types of mechanical scarification and a sulfuric acid scarification. Each treatment and a control (unscarified) were then split into two parts with or without 100 μM gibberellic acid. Seeds were tested in greenhouse, germination chamber and field trials. Field trials were planted in the fall of 1977 at the oil shale revegetation site in the Piceance Basin, western Colorado. Tetrazolium tests revealed viability of ungerminated seed and therefore amount of seed killed by the treatment. Germination percentage varied from 5 to 75 percent depending on year and treatment; the older the seed the better the germination in all trials. In greenhouse and germination chamber the acid treatment performed best, while in the field trial the mechanical treatments were best. All treatments responded to gibberellic acid with increased germination. Seedlings which germinated from the controls were set out for genetic recombination aiming toward a low dormancy strain.

-- ABSTRACT FORM -- 1978 AGRONOMY ABSTRACT --

The American Society of Agronomy

FORM B -- Complete one (1) copy. Follow Directions below. Indicate Division(s) C-4

TYPE ABSTRACT HERE

Seed Production Characters in Selection of Western Wheatgrass for Revegetation Uses. Walter W. Padilla, Robin L. Cuany*, and Glen P. Murray, Colorado State University.

Western wheatgrass (*Agropyron smithii* Rydb.) will be one species used in rehabilitation of disturbed oil-shale land in western Colorado, as well as other revegetation uses in plains and mountains. A variety specifically adapted to this use was sought from among the variability found in over 75 accessions planted in two spaced-plant nurseries at Fort Collins and Meeker. There were significant differences in rhizomatous spread, herbage rating, culm number, seed yield, 100-seed weight, spike length, and culm height. The correlations among herbage rating, culm number, and seed yield were high enough that either of the first two could be used as a selection tool for seed yield. Seed yield per plant at 1.5 m spacing averaged 6g but named varieties released by SCS ranged up to 17 g and individual selected plants reached 57 g. The 100-seed weights ranged from 377 to 951 mg but were uncorrelated with the parent plant spread. The best 100 plants are under progeny test in the oil-shale area study site. The significance of seed characters to success in establishment and suitability for revegetation use will be discussed.

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ROLE OF SOIL MICROORGANISMS AS INDICATORS AND POSSIBLE CONTROLLING FACTORS IN PLANT SUCCESSION PROCESSES ON RETORTED SHALE AND DISTURBED SOILS

Personnel: Dr. Donald A. Klein, Associate Professor
Dr. Larry E. Hersman, Research Associate
Mr. Nicholas Nagle, Research Technician
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OBJECTIVES

The overall objectives of this subproject are to determine the possible responses and changes in soil microbial types and activities during the reestablishment of plant communities on disturbed sites and, if possible, to develop management alternatives by which microbe-plant relationships can be controlled to enhance succession processes in disturbed soils. In addition, the effect of soil storage conditions on soil microbiological characteristics and the effects of retorted shale materials on essential soil microbiological processes are to be evaluated.

Specific objectives include the following:

Phase I: To evaluate microbial responses and changes in number and activity during reestablishment of plant communities on disturbed soils.

Job 1-1: Monitoring of soil microbiological processes on test subplots at the Intensive Study Site.

Job 1-2: Study the effects of soil storage on microbiological populations and on microorganism-related nutrient cycling processes.

Job 1-3: Determine the effects of retorted shale materials on the microbiological characteristics of surface soils from the study site.

Phase II: To develop approaches for controlling or modifying plant-microbe interactions.

Job 2-1: Evaluate the microbiological characteristics of plants being considered for use in planting mixtures with a major emphasis on the role of symbiotic nitrogen-fixing plants in stand establishment and the nitrogen status of the revegetation system.

Job 2-2: Study plant material decomposition effects on soil microbiological processes. This will be carried out using introduced and native species.

Job 2-3: Study effects of physical disturbance on the microbiological activies of soils.

PROGRESS TO DATE

Field Site Sampling

Revegetation Technique Plot

For the 1978-1979 season, samples were taken in May and July from subplots 1-24 and 122-144 (Figure 2). From each subplot three samples were taken at each of the ends and in the center of the rectangular area. The 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 would occur. Also, no aboveground plant materials or soils directly adjacent to plants were taken to maintain the experiment integrity of the plots.

Successional Plot

For this series of subplots three subsamples were taken from each individual plot in the manner described for the Revegetation Technique Plot. Samples were collected using a clean spade and stored in double-wrapped plastic bags at refrigerated temperature (6°C) until used in the laboratory.

Topsoil Storage Plot

Samples were collected from the Topsoil Storage Plot in August of 1978 using a 10 cm diameter soil corer. Four cores were taken across the storage pile at 0-51, 15-30, 30-61, 61-91, 91-122, and 122-152 cm depths. These subsamples were placed in individual plastic bags and immediately returned to the laboratory for analysis. The samples from each of these depth intervals were mixed prior to analysis.

Soil-Shale Mixture Experiments

Topsoil and Paraho retorted shale were collected at the Intensive Study Site for use in these studies.

Soil Sample Processing

Samples were sieved with a 2 mm mesh screen, mixed in a Patterson-Kelly, twin shell, dry blender for 20 minutes, returned to individual plastic bags and stored at 6°C until analysis or use in experiments.

Topsoil and retorted oil shale were mixed in a Patterson-Kelly, twin shel, dry blender to yield the following percentage by weight of shale-to-soil mixtures: no shale, 10 percent shale, 20 percent shale, 30 percent shale, and 100 percent shale. These mixtures were used as potting soils for greenhouse experiments, and these were also monitored for chemical microbial characteristics over varied incubation times.

Analytical Procedures

pH

Ten grams of soil and 90 ml of water were mixed together. The pH of the solution was determined with a Fisher accumett pH meter.

Percentage Moisture

Weighing pans were oven dried (105°C) for 24 hours and then dessicated for an additional 24 hours. Soil samples of 1 g were placed in the weighing pans, dried for 24 hours (105°C), placed in a dessicator for 24 hours, and weighed again. Percentage moisture was determined by dividing the weight difference of the wet and dry soil by the wet soil weight.

Percentage Organic Matter

Three-tenths of a gram of soil and 3 ml of 1 N potassium dichromate were placed into 35 ml bottles. Immediately, 6 ml of concentrated H_2SO_4 was added and the mixture was swirled for ten seconds. After the mixture was allowed to stand for ten minutes, 30 ml of deionized water was added. The bottles were again swirled for ten seconds and allowed to stand for an additional hour. The mixture was centrifuged at 5,000 rpm for ten minutes; the supernatant was decanted into cuvettes and read with a spectronic 20 colorimeter at 610 nm. The percentage organic matter was determined by comparison against a standard curve prepared with 2 and 4 percent organic matter reference soils.

Soil Microbial Enumeration Techniques

Fungi were enumerated using Rose Bengal Agar consisting of 10 mg of dextrose, 5 g peptone, 1 g monobasic potassium phosphate, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.033 g of Rose Bengal, 20 g of Agar, and 1,000 ml of deionized water. This was sterilized and, after cooling, 3 ml of strotomycin solution was added to the medium using procedures of Martin (1950). The actinomycetes and bacteria were enumerated with sodium caseinate agar consisting of 0.2 g sodium caseinate, 5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeCl_3$, 15 g agar, and 1,000 ml of H_2O.

Eleven grams of the sifted soil was added to a standard phosphate dilution blank (99 ml), shaken for 15 minutes on a reciprocal shaker at 125 rpm, diluted, and inoculated onto spread plates using 0.1 ml aliquots. Plates were incubated at room temperature for two weeks before being counted to assure maximum expression of colonies.

Nitrogen Fixation

Nitrogen fixation activities of soils and plants were carried out using procedures described by Hardy et al. (1968) and Hardy et al. (1973). Specific equipment used in the laboratory includes a Varian Aerograph Model 1200 flame ionization gas chromatograph and an integrator-recorder to allow calculation of peak areas. Acetylene and ethylene standards were obtained from the Applied Science Laboratories, State College, Pennsylvania. The separation of acetylene from ethylene was carried out by use of a 3 mm diameter x 133 cm length stainless steel column of Poropak Q (Water Associates, Milford, Massachusetts) with a column bath temperature of 70°C.

Ten grams of soil were placed in serum bottles and brought to 60 percent moisture holding capacity with a 0.5 percent solution of glucose in water. The bottles were sealed with serum caps and flushed with N_2 gas for five minutes. Using a 10 ml syringe, 5 ml of gas were withdrawn from each bottle, and 5 ml of acteylene was added using the gas washing train recommended by Hardy et al. (1973). The bottles were incubated in the dark for 48 hours at 25°C. Using a 1 ml syringe, 1 ml of gas was withdrawn and injected into a Varian gas liquid chromatograph. Nitrogen-fixation activity was expressed as nanomoles of ethylene produced 10 g $soil^{-1} \cdot 48\ hours^{-1}$.

Dehydrogenase Activity

One gram of soil, 0.2 ml of 3 percent triphenyl-tetrazolium chloride solution, and 0.5 ml of a 0.5 percent gulcose in water solution were placed in test tubes, mixed by shaking, and incubated at room temperature for 24 hours. Methanol (19 ml) was added to each tube which was mixed by inversion. The mixture was transferred to 50 ml centrifuge tubes and centrifuged at 12,000 rpm for ten minutes. The supernatants were decanted into cuvettes and read at 485 nm on a Spectronic 20. Dehydrogenase activity was recorded as μg formazan produced · gram $soil^{-1}$ · 48 $hours^{-1}$ as described by Klein et al. (1971).

Phosphatase Activity

Five-tenths gram of soil was placed in 50 ml centrifuge tubes with 2 ml of MUB buffer [Tris (hydroxymethyl) amino methane, 3.025 g; maleic acid, 2.9 g; citric acid, 3.5 g; boric acid, 1.57 g; 1 N NaOH, 122 ml; volume brought to 250 ml, pH 8.5], 0.125 ml of touene, and 0.5 ml of 0.115 n paranitrophenylphosphate (Skujins et al. 1962). The tubes were swirled, stoppered, and incubated for one hour at 37°C. Then, 0.5 ml of 0.5 N CaCl and 2 ml of 0.5 n NaOH were added; the mixtures were swirled and centrifuged at 5,000 rpm for ten minutes. Supernatants were decanted into cuvettes and read at 400 nm on a Spectronic 20. Phosphatase activity was determined by comparison of these values to a standard curve constructed utilizing various concentrations of paranitrophenol (0 to 50 μg per ml) and expressed as μg of paranitrophenol released · gram $soil^{-1}$ · $hour^{-1}$ (Tabatabai and Bremner 1969).

Adenosine Triphosphate (ATP) Levels

ATP levels in soils were determined using the basic procedures of Paul and Johnson (1977). Ten grams of soil, 100 ml of 0.2 n $NaHCO_3$ (pH 8.5), and 10 ml of $CHCl_3$ were mixed in a Waring blender for one minute; an additional 100 ml of 0.2 N $NaHCO_3$ was added; and the mixture was blended for one additional minute. Ten ml of this slurry was pipetted into a 50 ml centrifuge tube and centrifuged for 10 minutes at

12,00 rpm. Five ml of supernatant was pipetted into 25 ml volumetric flasks which were then heated in a 60°C bath for 20 minutes. The volume was brought to 25 ml by the addition of Tris buffer (pH 7.8). The flasks were mixed by inversion, and 5 ml of this mixture was pipetted into 10 ml test tubes which were immediately frozen with a mixture of dry ice and acetone. These frozen extracts were stored at 5-10°C until analysis. ATP analyses were performed using purified DuPont luciferin-luciferase enzymes and an Aminco chemglow photometer. ATP concentrations were determined by comparison of sample values to values obtained from standard curves with ATP concentrations ranging from 10^{-9} to 10^{-12} μg ATP ml^{-1}. Values were reported as ng ATP/gram soil.

^{14}C Glucose Mineralization

Folder filter paper strips were placed in suspended traps which were previously attached to serum caps using the basic procedure of Harrison et al. (1970). Using serum bottles, 7 ml of substrate mixture (5 ml K_2HPO_4 - KH_2PO_4, 0.05 M, pH 7.4; 2 ml of unlabeled glucose substrate consisting of 5, 10, 20, 30, 40, or 50 μg per bottle) and 50 μl radiolabeled glucose substrate (0.1 μ Ci) were added. To the control bottle, 1 N H_2SO_4 was added. At 30 second intervals 0.3 g of soil were added to the remaining reaction bottles; each was then capped and placed on the shaker. After 20 minutes, 1 ml of 1 N H_2SO_4 was added in order to each reaction bottle at 30 second intervals. The bottles were placed on a shaker for ten minutes, and then 0.15 ml of phenylethylamine was added to each suspended trap using a 1 ml syringe. The reaction bottles were place on a shaker for one hour.

After one hour the filter papers were removed from the suspended traps and placed in individual scintillation vials containing 10 ml of liquid scintillation fluid (ICN, Irvine, California). The vials were then placed in a Beckman LS-133 liquid scintillation counter, and $^{14}CO_2$ raw counts were recorded. One hundred percent efficiency $^{14}CO_2$ counts were obtained by multiplying the raw $^{14}CO_2$ counts by the product of the external ratio and the glucose quench curve.

Plant-Associated Nitrogen Fixation Potential

Ladak alfalfa, sainfoin (Northrup King, Longmont, Colorado) and western wheatgrass (Botany Department, CSU, Fort Collins, Colorado) were grown in the previously described soil-retorted shale mixtures to investigate possible effects of retorted shale on plant-associated nitrogen fixation. Seeds for both leguminous plants were inoculated as recommended by Northrup King using the inoculum purchased with the seeds. Approximately 25 seeds were placed in 3.85 l pots containing premoistened soil-shale mixtures (one species per pot) and covered with 1.0-1.5 cm of the same soil-retorted shale mixture. Two such planting experiments were performed two weeks apart in triplicate. After sprouting the total number of plants per pot was reduced to 16 to insure adequate space for optimal growth. Initial pot positions in the greenhouse were statistically randomized and then rotated three times per week. Soil moisture was maintained at approximatly 60 percent of field-moisture holding capacity; diurnal conditions were natural, while greenhouse temperatures were monitored daily.

After six weeks of incubation the plants were harvested by washing away the soil. Above- and belowground biomass, number of nodules per gram dry root, and acetylene reduction values were determined. For the acetylene reduction determination the following procedure was employed:

1. Within one hour after harvesting all the roots from one pot were placed in one 60 ml stoppered serum bottle.
2. Using a 5 ml syringe 5 ml of serum bottle atmosphere was replaced with 5 ml of acetylene.
3. Serum bottles were incubated in the dark for 24 hours at 25°C.
4. Ethylene production was determined as described previously for nitrogen fixation.
5. Ethylene production was recoreded a n Mol ethylene reduced per gram dry root.

Nonplant-Associated Nitrogen Fixation Potential

Soils were mixed with retorted shale or with sterile glass beads (5 mm diameter) to evaluate the relative effects of soil dilution with retorted shale on microbial functions and assayed for nitrogen fixation potential using previously described procedures.

Shale Extraction Experiments

Soils were mixed with retorted shale which had been exposed to methylene chloride for 48 hours, washing for 24 hours with methyl alcohol, and washed for 24 hours with double distilled sterile water. The purpose of this experiment was to determine whether the organic or inorganic fraction of retorted shale was responsible for the previously reported effects of retorted shale on microbial activities (Hersman and Klein 1978).

Effects of Retorted Oil Shale Water and Incubation with Helium or Nitrogen Gas Mixtures on Plant-Associated Nitrogen Fixation Potential

The following procedure was employed to determine what effects retorted oil shale water has on the nitrogen fixation process of Ladak alfalfa, using atmospheres of oxygen with helium or nitrogen.

1. Root nodules were collected from six-week-old alfalfa plants.
2. Root nodules and roots without nodules (control) were placed in stoppered 23 ml serum bottles.
3. Sixty ml of water was mixed with 30 g of retorted oil shale for three hours. Using a sterile syringe 0.5 ml of this water was added to half the serum bottles while 0.5 ml of sterile water was added to the remaining bottles.
4. Half of the sealed serum bottles were purged with a gas mixture of 21 percent oxygen and 79 percent helium for ten minutes, after which .03 ml of CO_2 was added. An ambient atmosphere was maintained for the remaining serum bottles.
5. Using a 5 ml syringe, 2.3 ml of serum bottle atmosphere was replaced with 2.3 ml of acetylene.
6. Serum bottles were incubated in the dark for 17 hours at 25°C.
7. Ethylene production was measured using gas-liquid chromatography as described previously and recorded as m moles of ethylene produced per ml serum bottle atmosphere.

Statistical Analysis

All data were analyzed by standard statistical procedures, including analysis of variance and regression analysis using available computer programs.

Results

Revegetation Technique Plot

For the Revegetation Technique Plot which was sampled both during May and July of 1978 irrigation, seeding methods, seed mixtures, and fertilizer levels showed significant effects (single and interactive) on soil microbiological processes (Tables 42-44). For both the May and July samplings strong overall direct correlations were shown between the percentage soil organic matter and pH (inverse), soil water, dehydrogenase, phosphatase activity, N_2 fixation potential, and soil ATP which appeared to be related to plant growth with subsequent organic matter release to the soil. With irrigation the native seed mixture showed the highest levels of soil organic matter, while without irrigation the introduced species mixture was related to the highest organic matter content in the test soils. This would suggest that different seed mixtures may be appropriate for use in irrigated versus nonirrigated revegetation programs if belowground production is the primary concern. In addition, a trend towards decreased N_2 fixation potential with fertilizer nitrogen additions was noted in comparisoin with the non-fertilized plots for the spring sampling at a significance level of $P = <.06$.

For the May 1978 sampling, excellent Pearson correlations were noted between measurements (Table 42). Generally, the pH changes showed inverse relationships with parameters related to general microbial activity and organic matter accumulation by the test plants. In contrast, the changes in soil moisture and organic matter with other general microbiological parameters showed good direct correlations. These

Table 42. Revegetation Technique Plot Pearson correlations--May 1978 sampling.

Parameters	Related Parameters	Significance[1]
pH	Dehydrogenase	** (-)[2]
	Phosphatase	** (-)
	N_2 fixation	** (-)
	Organic matter	** (-)
Soil water	pH	* (-)
	ATP	* (-)
	Organic matter	*
Dehydrogenase	Phosphatase	**
	N_2 fixation	**
	Organic matter	**
Phosphatase	N_2 fixation	**
	ATP	**
	Organic matter	**
N_2 fixation	ATP	**
	Organic matter	**

[1]** = 1 percent significance level; * = 5 percent significance level.

[2](-) = inverse correlation.

Table 43. Revegetation Technique Plot Pearson correlations--July 1978 sampling.

Parameters	Related Parameters	Significance[1]	
pH	Soil water	**	(-)[2]
	Phosphatase	**	(-)
	N_2 fixation	**	(-)
	ATP	**	(-)
	Organic matter	**	(-)
Soil water	Phosphatase	**	
	N_2 fixation	**	
	ATP	**	
	Organic matter	**	
Dehydrogenase	Phosphatase	*	
	Organic matter	**	
Phosphatase	N_2 fixation	**	
	ATP	**	
	Organic matter	**	
N_2 fixation	ATP	**	
	Organic matter	**	
ATP	Organic matter	**	

[1]** = 1 percent significance level; * = 5 percent significance level.

[2](-) = inverse correlation.

Table 44. Significant analysis of variance interaction for the Revegetation Technique Plot for May and July samplings.[1]

Test Variable	May Sampling		July Sampling	
	Effect	Significance	Effect	Significance
pH	Irr.	**	Irr.	**
	Mix.	**		
	Irr./Mix.	**		
Soil water	None		Irr.	**
			Fert.	*
			Tech.	**
			Mix.	*
Dehydrogenase	Irr.	**	Irr.	**
Phosphatase	Irr.	**	Irr.	**
	Mix.	*	Mix.	**
N_2 fixation	Irr.	**	Irr.	**
	Fert.	*		
	Irr./Mix.	*		
ATP	None		Irr.	**
Organic matter	Irr.	**	Irr.	**
	Mix.	**	Mix.	**
			Irr./Mix.	*

[1]** = 1 percent significance level; * = 5 percent significance level.

results suggest that under routine management that it may be possible to utilize fewer parameters to monitor the reestablishment of these essential microbiological processes.

A similar set of relationships was observed for the July 1978 sampling, as noted in Table 43. Especially important is the strong set of correlations between soil organic matter changes and general microbiological activity parameters

The analyses of variance for both the May and July samplings provided additional information concerning the effects of management variables on microbiological processes. A summary of the significant relationships suggested by the analyses of variance is given in Table 44. Several observations are of interest in the analysis of these reponses. For both the May and July samplings irrigation had significant independent effects upon soil pH, dehydrogenase and phosphatase activities, ATP levels, and organic matter levels in the surface soils. As irrigation did not begin until after the May sampling, irrigation did not share a relationship with the soil water content. These results indicate that the irrigation effect on these microbiological parameters resulted from the previous year's irrigation activity. A major point of interest from both of these samplings was the effect of seed mixture on the surface soil organic matter dyanmics, which was independent of fertilization and treatment variables which were used in this test plot for both May and July (Figures 48 and 49, respectively). These results suggest that the native plant species mixture may be able to show better soil organic matter accumulation with irrigation, while without irrigation the introduced plant species mixture may be able to allow better organic matter accumulation in the surface soil. In comparison, for either of the

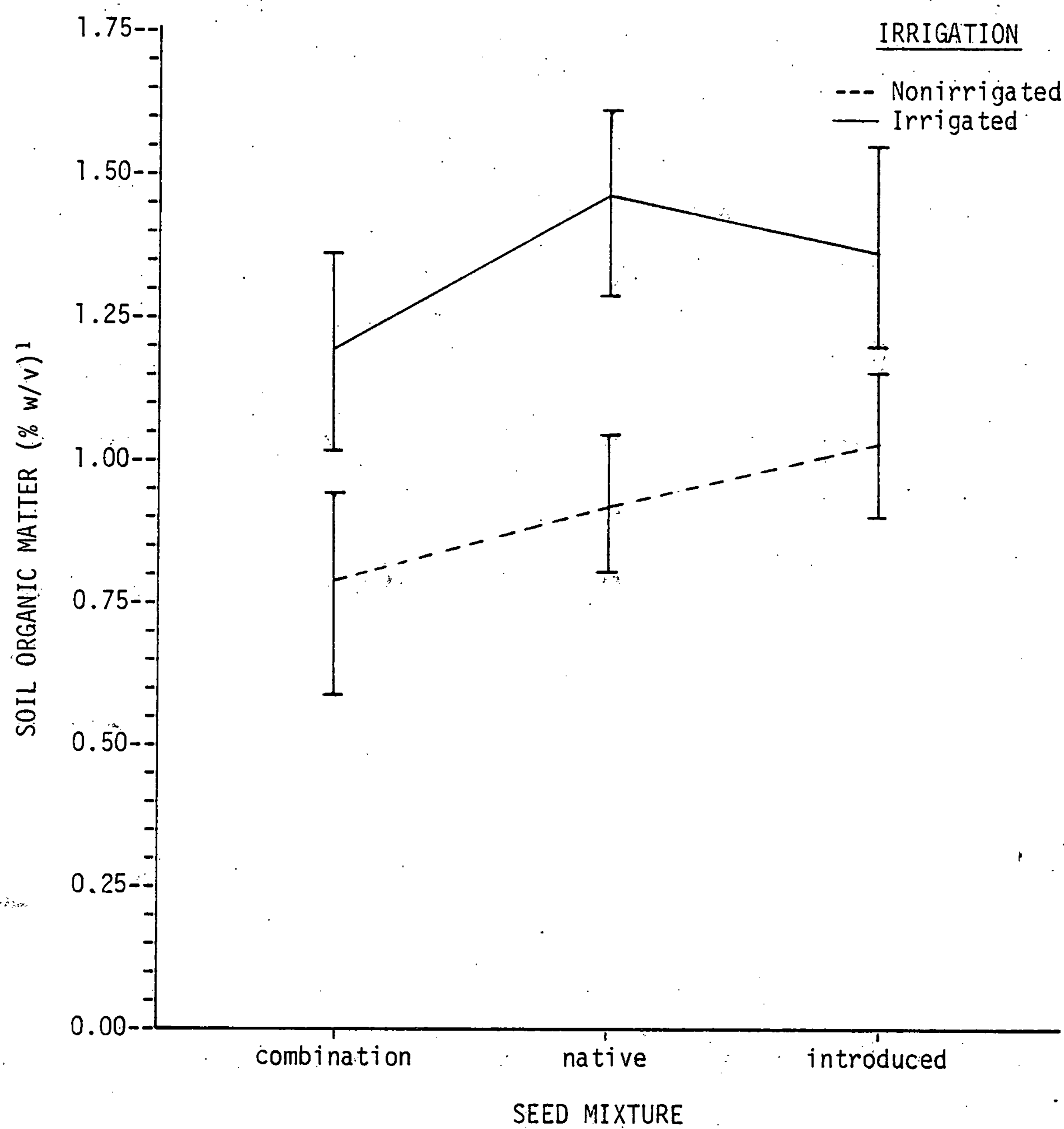

Figure 48. Irrigation and seed mixture effects on surface soil organic matter levels--May 1978 sampling on Revegetation Technique Plot. (Standard deviations are shown.)

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[1]Values corrected based on subsequent standard readjustment.

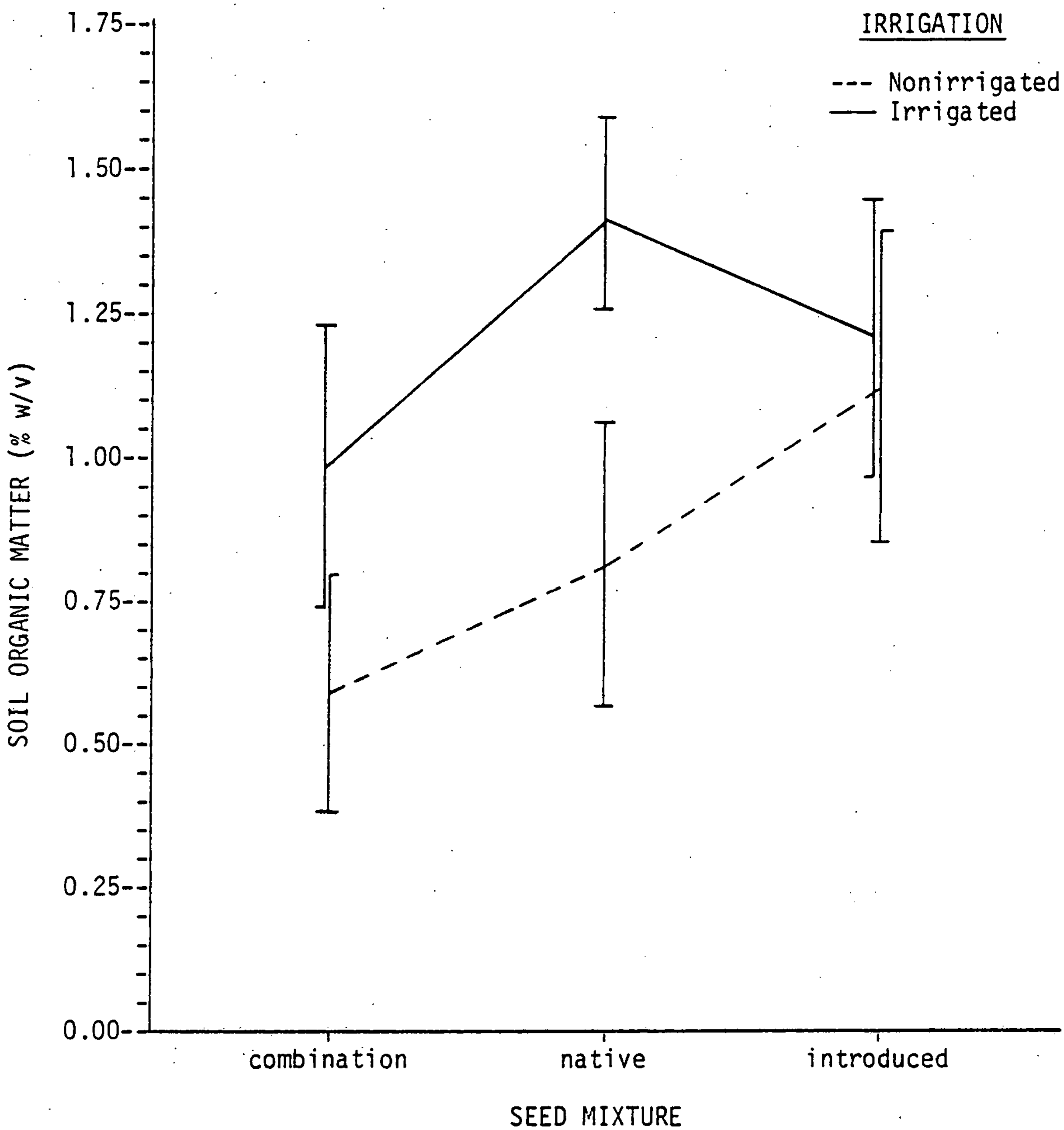

Figure 49. Irrigation and seed mixture effects on surface soil organic matter levels--July 1978 sampling on Revegetation Technique Plots. (Standard deviations are shown.)

sampling times and with or without irrigation the combination mixture did not lead to similar organic matter accumulation.

Together with these changes in organic matter accumulation which appear to be related to planting methods and irrigation variables, corresponding changes were also observed for several of the other microbiological parameters including phosphatase and dehydrogenase activity and nitrogen fixation.

The data for the dehydrogenase and phosphatase responses are summarized in Tables 45 and 46, respectively. For both the May and July samplings these data also illustrate that without irrigation the introduced grass species will tend to allow more rapid development of biogeochemical cycling processes, while with irrigation the native species mixture will provide equivalent or slightly higher activities. For the spring sampling (May) fertilization was related to decreased phosphatase activity ($P=\leq 6.5$ percent) which was not observed for the July sampling.

Nitrogen fixation activity also showed changes which may have applications in the development of revegetation management techniques. With irrigation higher but not significantly different nitrogen fixation rates were noted in comparison with the nonirrigated plots. In addition, a trend (nonsignificant) towards decreased nitrogen fixation on the fertilized plots also was noted for the May sampling which was not observed in July. Thus, for both nitrogen fixation and phosphatase decreased activities in relation to N and P additions may have occurred in the spring, while this trend was not noticed for the July 1978 sampling.

Table 45. Dehydrogenase activity relationship to irrigation and seed mixture--May and July 1978 samplings for the Revegetation Technique Plot. (Formazan, $\mu g \cdot gram\ soil^{-1} \cdot 48\ hours^{-1}$).

Sampling Time	Irrigated	Seed Mixture[1]		
		Mixed	Native	Introduced
May	yes	14.0 ± 3.3	18.9 ± 6.4	15.8 ± 4.7
	no	2.8 ± 4.8	1.2 ± 2.9	7.1 ± 7.3
July	yes	18.0 ± 2.0	15.9 ± 3.7	18.0 ± 4.5
	no	9.1 ± 5.1	17.5 ± 9.8	14.6 ± 7.5

[1]Means and standard deviations are given.

Table 46. Phosphatase activity relationship to irrigation and seed mixture--May and July samplings for the Revegetation Technique Plot. (Nitrophenol released · gram $soil^{-1}$ · $hours^{-1}$.)[1]

Sampling Time	Irrigated	Seed Mixture		
		Mixed	Native	Introduced
May	yes	111 ± 52	138 ± 21	142 ± 44
	no	54 ± 17	74 ± 27	124 ± 17
July	yes	111 ± 19	153 ± 16	147 ± 34
	no	72 ± 28	84 ± 22	117 ± 26

[1]Means and standard deviations are given.

Surface Disturbed Successional Plot

All subplots were sampled in triplicate for analysis, and the seed mixture had the major effect on the accumulation of organic matter in the soils ($F=3.339$, $P=<.009$) with the introduced grass mixture and the native grass-forb-shrub mixture giving the highest soil organic matter levels, both with and without mulching (Table 47). This response was independent of fertilization treatments. In addition, with higher soil water contents the soils tended to have higher pH values.

The overall Pearson correlations between parameters for this successional plot are shown in Table 48. In this plot which was not irrigated strong relationships were also shown between organic matter accumulation and other biogeochemical cycling parameters. Of interest were the series of inverse relationships between nitrogen fixation and phosphatase activity and between the nitrogen fixation rates and variations in surface soil organic matter levels.

The analysis of variance relationships for the plot are summarized in Table 49. Soil water changes showed interesting relationships with mulch, fertilizer, and seed mixtures, while the seed mixture showed 1 percent level relations with organic matter variations and a 6.6 percent level of significance for the relationship with phosphatase activity.

The nitrogen fixation measurements also showed trends which were similar to those shown for the Revegetation Technique Plot discussed previously in that with added mineral nitrogen a decreased nitrogen fixation potential was obsered, although this was not statistically significant. Considering all planting mixtures the controls (#3) had ethylene production rates of 36±20 n mol of ethylene $\cdot$ 10 g soil^{-1} $\cdot$ 48 hours^{-1}, while for high N-P and low N-P treatments (#1 and #2) the

Table 47. Effect of planting mixture and mulching on soil organic matter levels--1978 sampling for the Surface Disturbed Successional Plot.

Seed Mixture	Soil Organic Matter Level[1]			
	Mulched		Unmulched	
1. Native grass	0.71	0.55	0.87	0.70
2. Introduced grass	1.49	0.24	1.42	0.23
3. Native grass-forb	0.72	0.55	0.88	0.67
4. Introduced grass-forb	1.01	0.77	0.90	0.69
5. Native grass-forb-shrub	1.39	0.31	1.34	0.25
6. Native and Introduced grass-forb-shrub	0.96	0.74	1.00	0.76

[1]Percent w/w organic matter in soil. Standard deviations are given. Values corrected by reference with standard soils.

Table 48. Pearson correlation coefficient summary--1978 analyses for the Surface Disturbed Successional Plot.

Parameter	Related Parameters	Significance[1]	
pH	Soil water	**	
	Phosphatase	**	(-)[2]
	N_2 fixation	**	(-)
	Organic matter	*	(-)
Soil water	Organic matter	*	(-)
Dehydrogenase	N_2 fixation	*	
Phosphatase	N_2 fixation	*	(-)
	Organic matter	**	
N_2 fixation	Organic matter	**	(-)

[1]** = 1 percent significance level; * = 5 percent significance level.

[2](-) = inverse correlation.

Table 49. Significant analysis of variance interactions in the Surface Disturbed Successional Plot--1978 sampling.

Test Variable	Relationship	Significance[1]
Soil water	Mulch	**
	Fertilizer	**
	Mixture	**
Phosphatase	Mixture	6.6%
N_2 fixation	Mulch	6.0%
Organic matter	Mixture	**

[1]** = 1 percent significance level; * = 5 percent significance level.

values were 29±15 and 33±16 nanomoles, respectively. Similar slight decreases in phosphatase activity were noted for the subplots which had received inorganic phosphorus treatments.

Stored Soil Analyses

Although only a single complete analysis has been completed on the stored soil plot which was installed at the Intensive Site in May 1978, this has involved soil storage for approximately four months which may reflect changes which might occur under shorter-term operational soil storage conditions. During this time no plant cover had been established.

The Pearson coefficient correlations between the parameters examined in this study are summarized in Table 50. The strong direct relationship between the soil organic matter levels and microbiological activities is again evident. The analysis of variance relationships are summarized in Table 51. The major effects on activities and general soil characteristics appear to be related to the depth of soil storage, while some degree of variation between the bores did occur. For the bacterial and actinomycete populations, significant variations were observed between sample bores which were independent of sample depth.

The effects of soil storage depths on specific parameters are summarized in Figures 50-53. The soil samples at the surface of the storage pile were found to have significantly lower water contents than for those from the deeper locations, which would be expected (Figure 50). Together with this marked decrease in the water content of soils stored near the surface other changes were noted. The surface soil had significantly lower organic matter levels (Figure 51) which was noted to approximately the 61 cm level. Together with these changes the pH and nitrogen

Table 50. Pearson correlation coefficient summary--1978 analysis for the Stored Soil Stockpile.

Parameter	Related Parameter	Significance[1]	
Soil water	N_2 fixation	*	
	Bacteria	*	
pH	Phosphatase	**	(-)[2]
	Organic matter	**	(-)
	Glucose mineralization		
N_2 fixation	ATP	**	
	Organic matter	*	
ATP	Phosphatase	**	
	Organic matter	**	
	Glucose mineralization	*	(-)
	Fungi	*	(-)
Dehydrogenase	ATP	**	
	Phosphatase	**	
	Organic matter	*	
	Glucose mineralization	**	(-)
	Fungi	**	
Phosphatase	Organic matter	**	
	Glucose mineralization	**	(-)
Organic matter	Glucose mineralization	**	(-)
	Fungi	**	
Glucose mineralization	Actinomycetes	*	(-)

[1]** = 1 percent significance level; * = 5 percent significance level.

[2](-) = inverse correlation.

Table 51. Significant analysis of variance interactions--1978 sampling for the Stored Soil Experiment.

Test Variables	Relationship	Significance[1]
N_2 fixation	depth	7.3%
Organic matter	depth	*
Actinomycetes	bore	*
Bacteria	bore	*
pH	depth	*
Soil water	depth	**

[1]** = 1 percent significance level; * = 5 percent significance level.

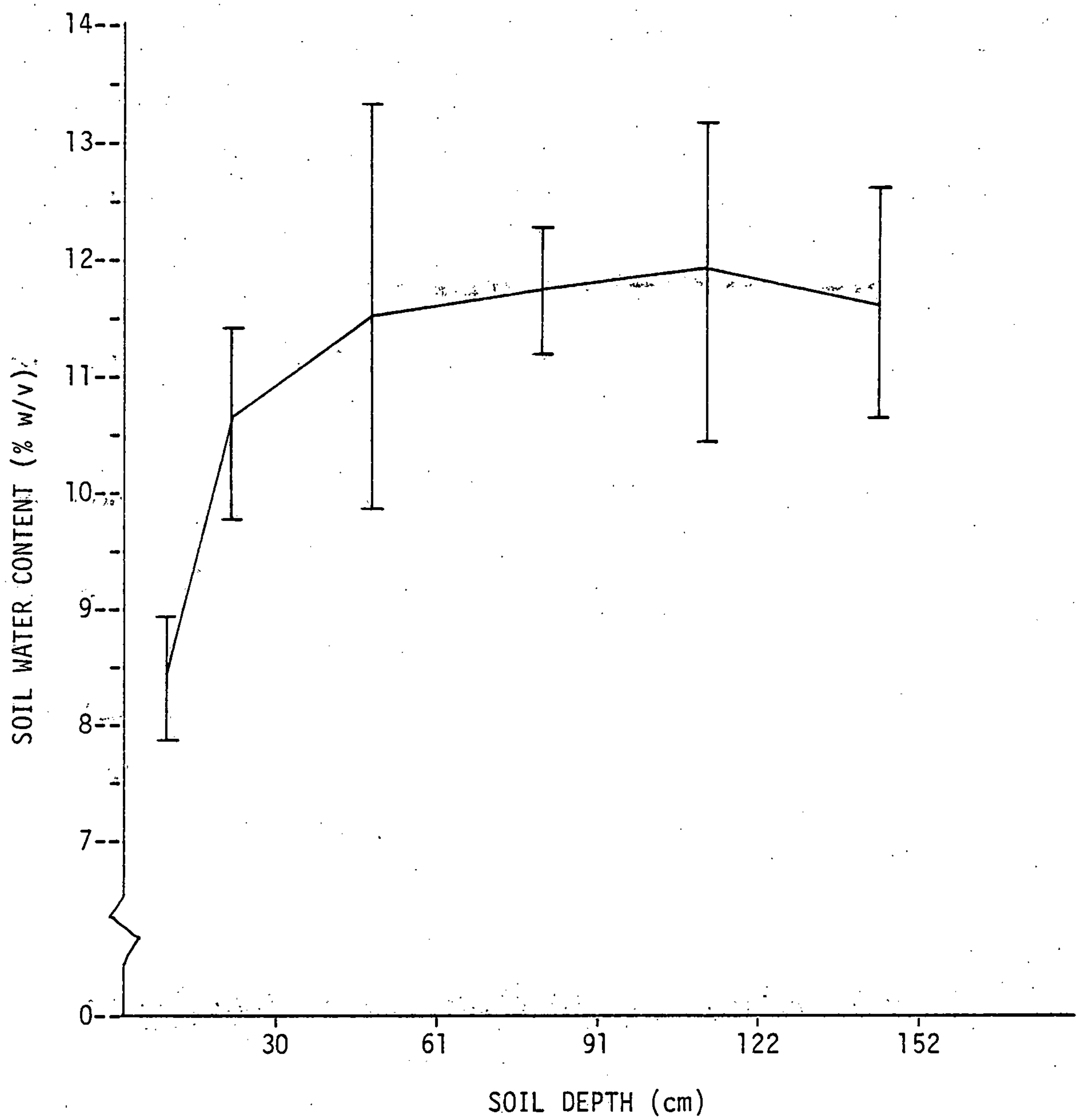

Figure 50. Depth effects on soil water content--1978 sampling from the stored soil experiment. (Standard deviations are shown.)

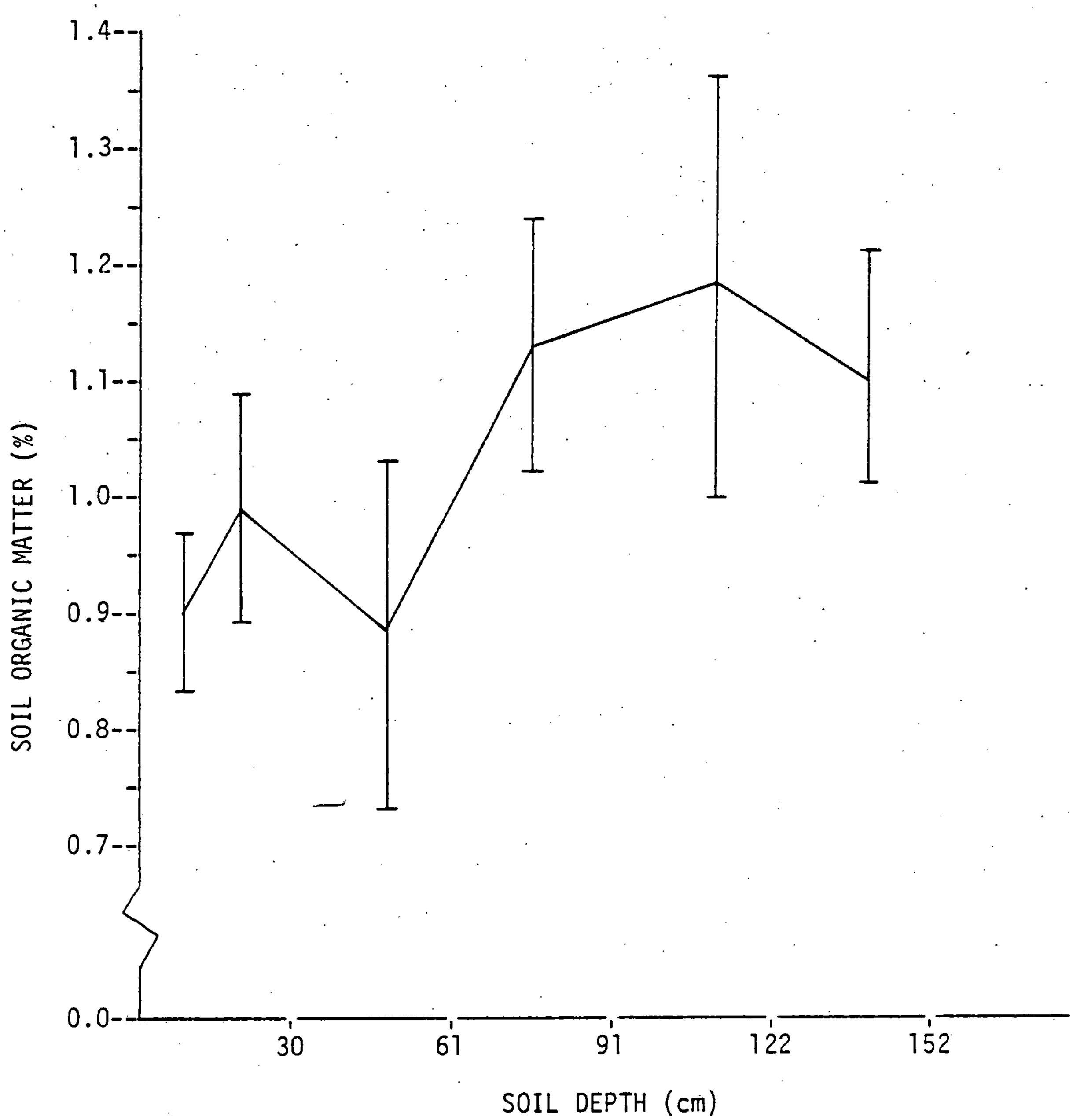

Figure 51. Soil depth effects on organic matter relationships for the stored soil experiment. (Standard deviations are shown. Values corrected based on subsequent standards readjustment.)

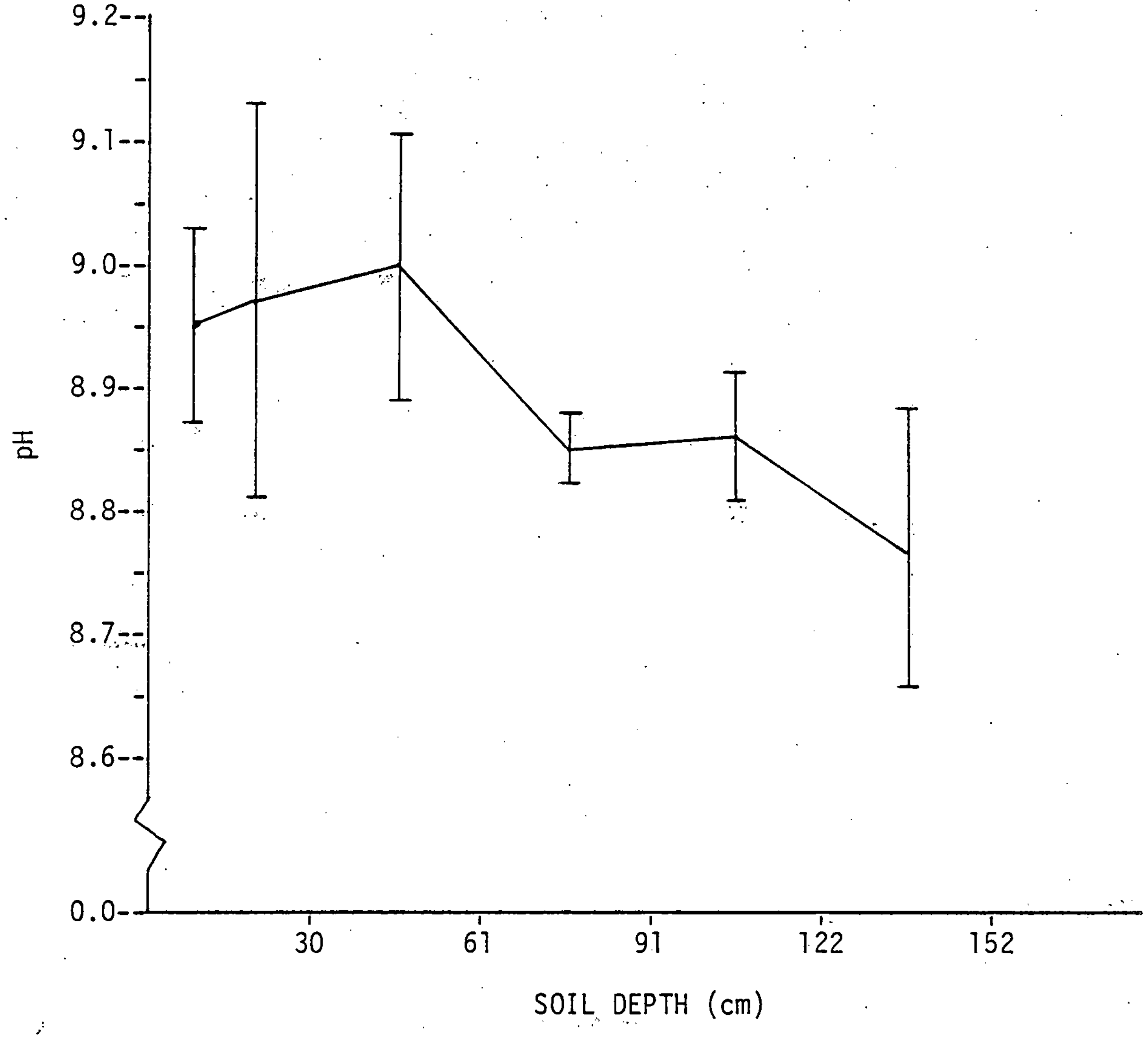

Figure 52. Depth effects on soil pH--1978 sampling from the stored soil experiment. (Standard deviations are shown.)

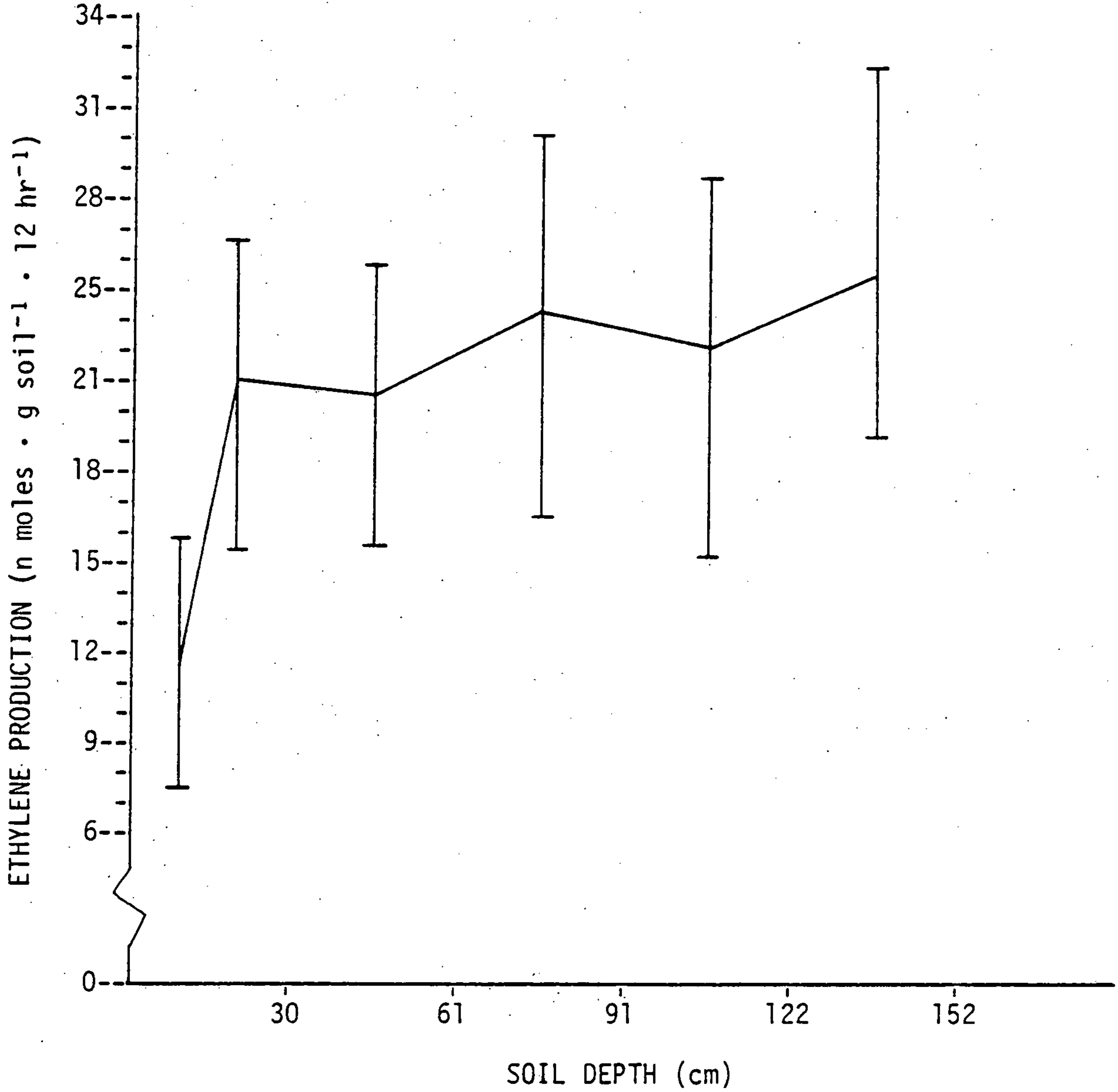

Figure 53. Depth effects on N_2 fixation--1978 sampling from the stored soil experiment. (Standard deviations are shown.)

fixation potential assays showed interesting trends. The three surface soil samples showed higher pH values than those given for the subsurface samples (Figure 52) which was a difference significant at the 1.8 percent level, and the nonplant-associated nitrogen fixation potential at the surface soil layer also was decreased (Figure 53) although this was not statistically significant. Together with these changes the surface soils sample (0-15 cm) also had lower bacterial and actinomycete populations although these changes were not signficant.

Effect of Retorted Shale on Plant-Associated Nitrogen Fixation

Two experiments were carried out during the research period concerned with the possible effects of retorted oil shale on nodulation efficiency and nitrogen fixation potential of two test legumes which were compared with western wheatgrass. In contrast with earlier studies of *Azotobacter*-type nonplant-associated nitrogen fixation, which was found to be sensitive to these materials (Hersman and Klein 1977, 1979), legume nitrogen fixation may not be as sensitive to these materials, and some degree of nitrogen fixation simulation has also been noted.

Generally, aboveground plant biomass tended to decrease with increasing retorted oil shale concentrations (Figure 54) for the three plants which were tested in this experiment. This decrease appeared to represent a direct relationship. In comparison the belowground biomass for the three plants decreased with 10 and 20 percent retorted shale additions, and no further general decreases were noted (Figure 55). In addition, a wider range of responses were noted for the belowground biomass of the three plants. Sainfoin showed only a minor response to

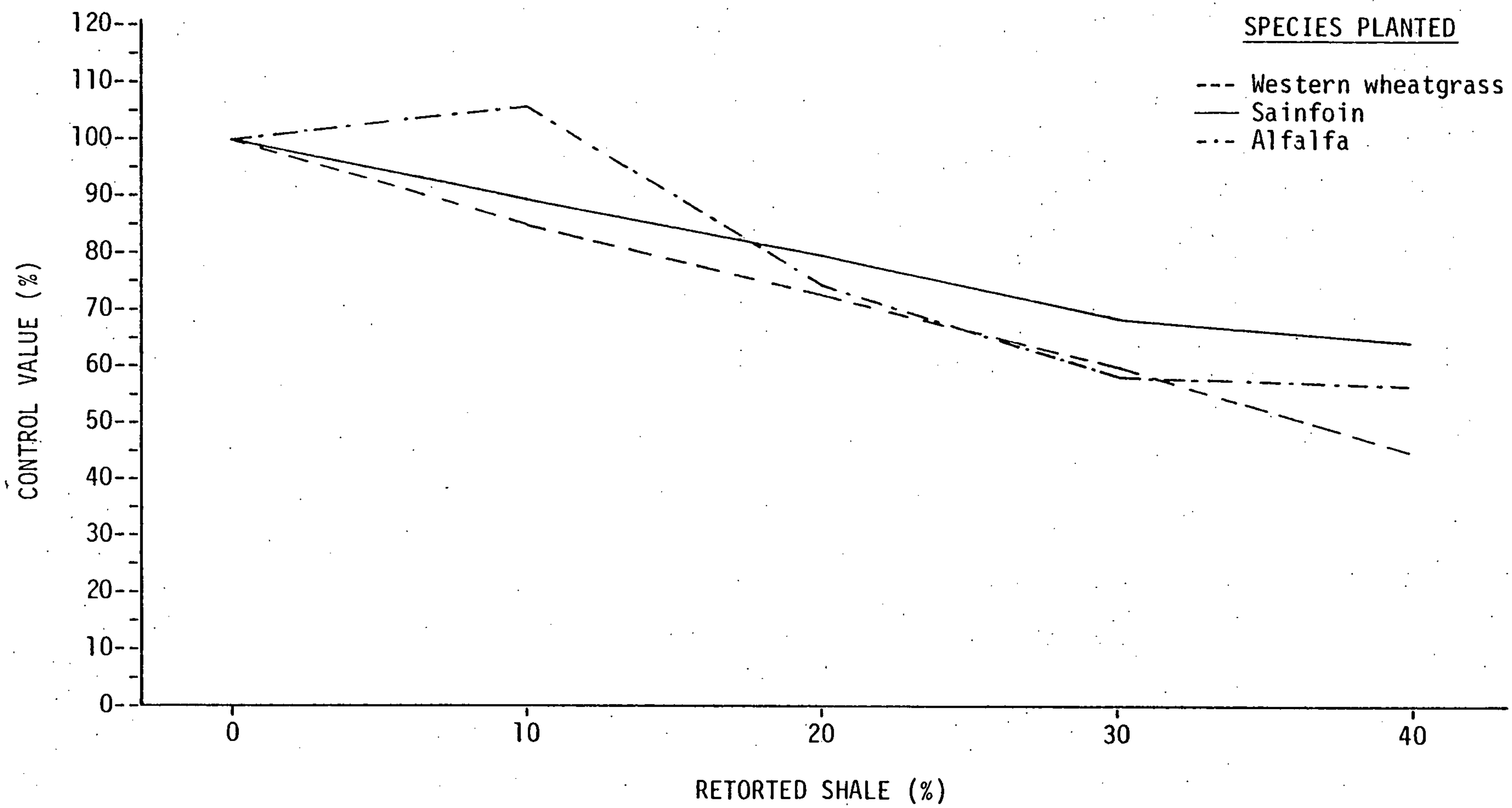

Figure 54. Changes in aboveground biomass for three species planted in soil-retorted shale potting mixtures.

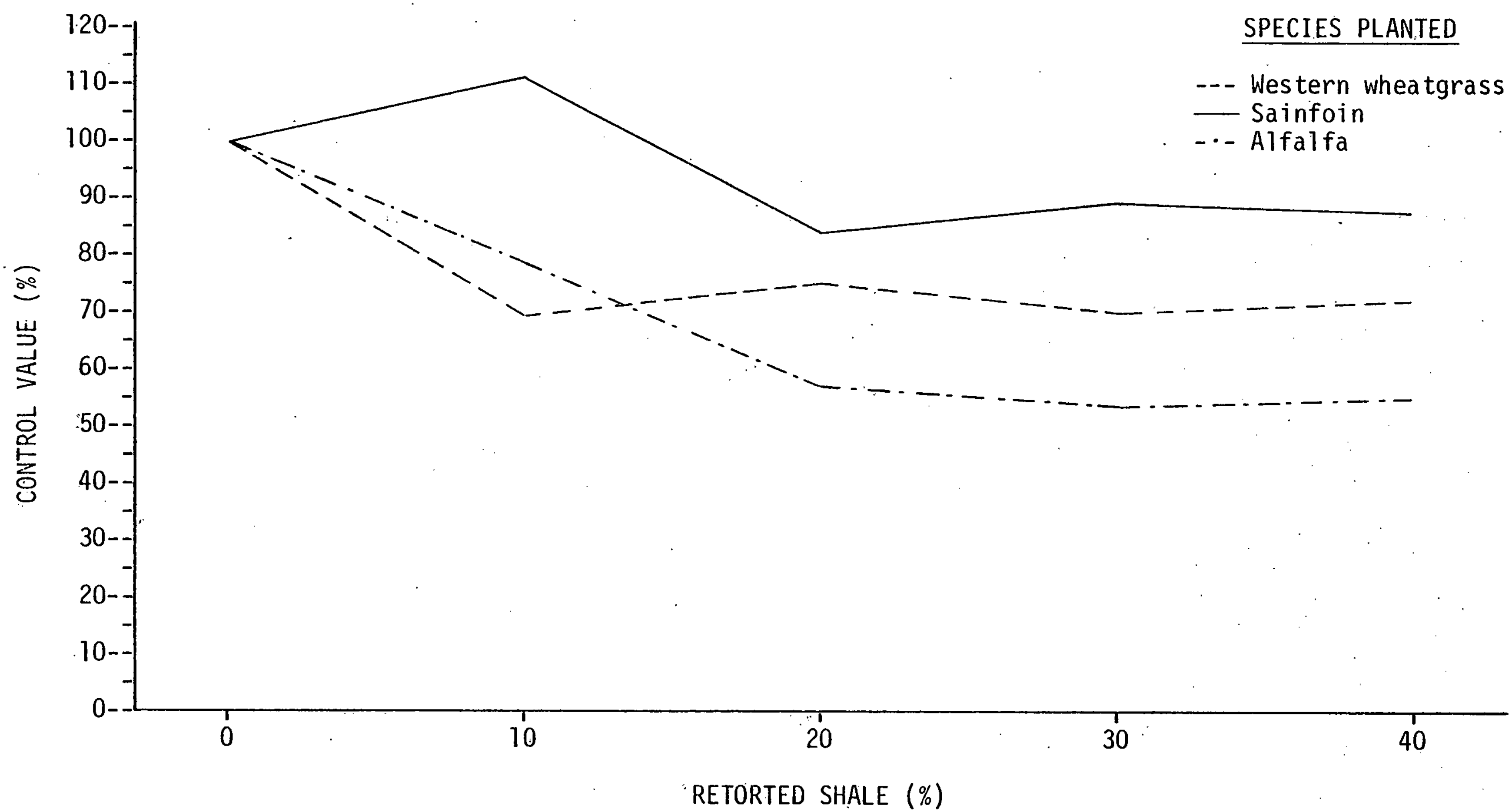

Figure 55. Changes in belowground biomass for three species planted in soil-retorted shale potting mixtures.

the added shale, while the western wheatgrass and alfalfa showed more distinct responses.

The number of nodules per gram dry root demonstrated an interesting response to the presence of retorted oil shale. With 10 percent retorted oil shale a decrease in nodules per gram dry root was observed, then with increasing concentrations of retorted oil shale general increases in the number of nodules for both sainfoin and Ladak alfalfa were observed (Figure 56). Similarly, nitrogen fixation responses showed initial declines, then increased with increasing concentrations of retorted oil shale (Figure 57).

Nonplant-Associated Nitrogen Fixation

Based on earlier studies where *Azotobacter*-type nitrogen fixation (nonplant associated) was found to be sensitive to the presence of retorted oil shale, additional experiments were carried out to determine if this effect might be due to simple dilution of the soil by the added oil shale or if this effect might be due to soluble shale components. For this purpose the nitrogen fixation (acetylene reduction) potential of a soil was measured with dilution by retorted shale, retorted shale extracted with methylene chloride, and by glass beads as an inert control (Figure 58).

A slight decline in the acetylene reduction potential was noted when the soils were diluted with glass beads, but in comparison the soils diluted with the retorted oil shales (either with or without methylene chloride extration) showed further distinct decreases in acetylene reduction potential. These results indicate that the effect of retorted shale on these nitrogen fixation processes is not due merely to soil

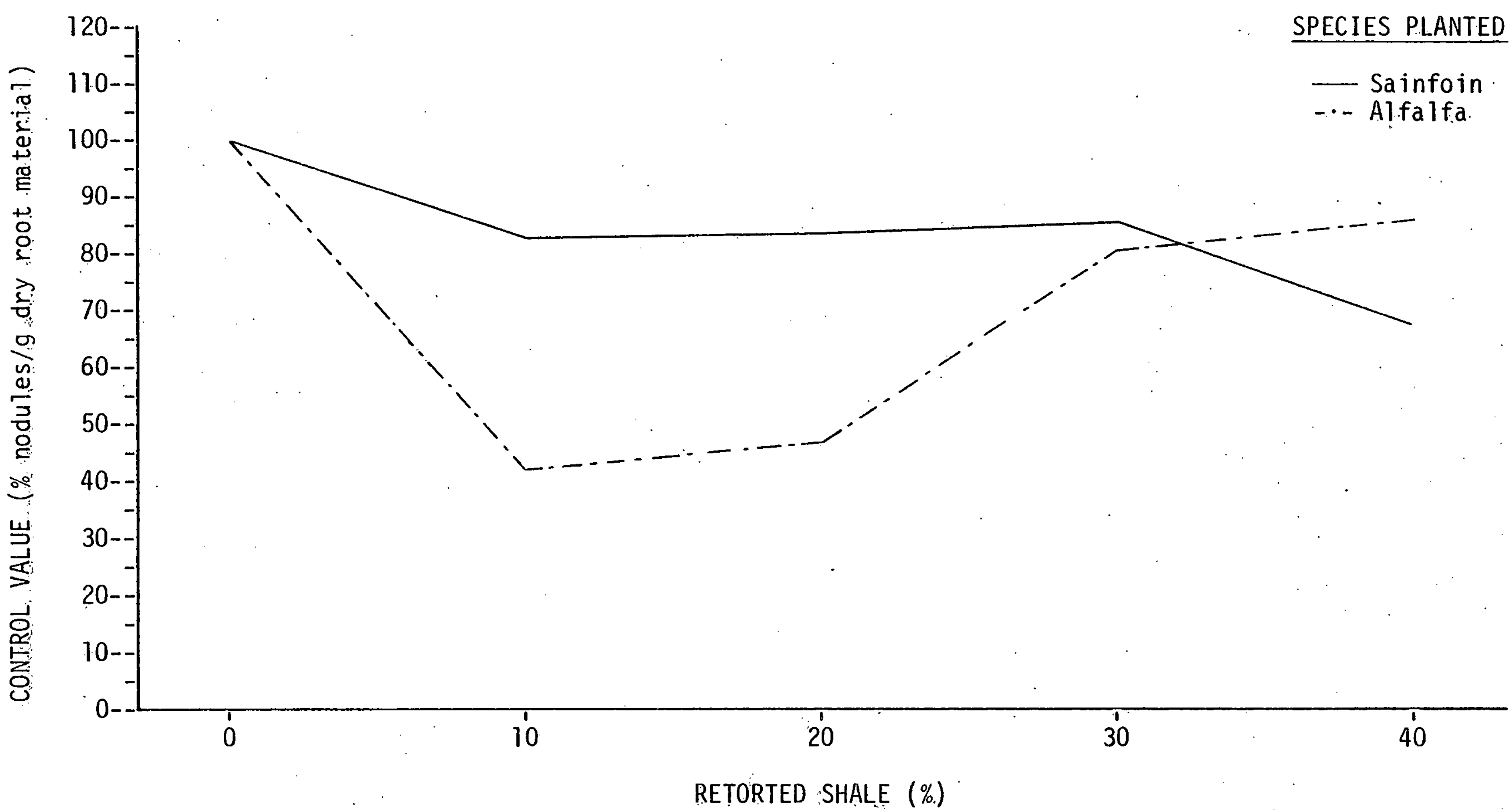

Figure 56. Changes in number of nodules for sainfoin and alfalfa planted in soil-retorted shale potting mixtures.

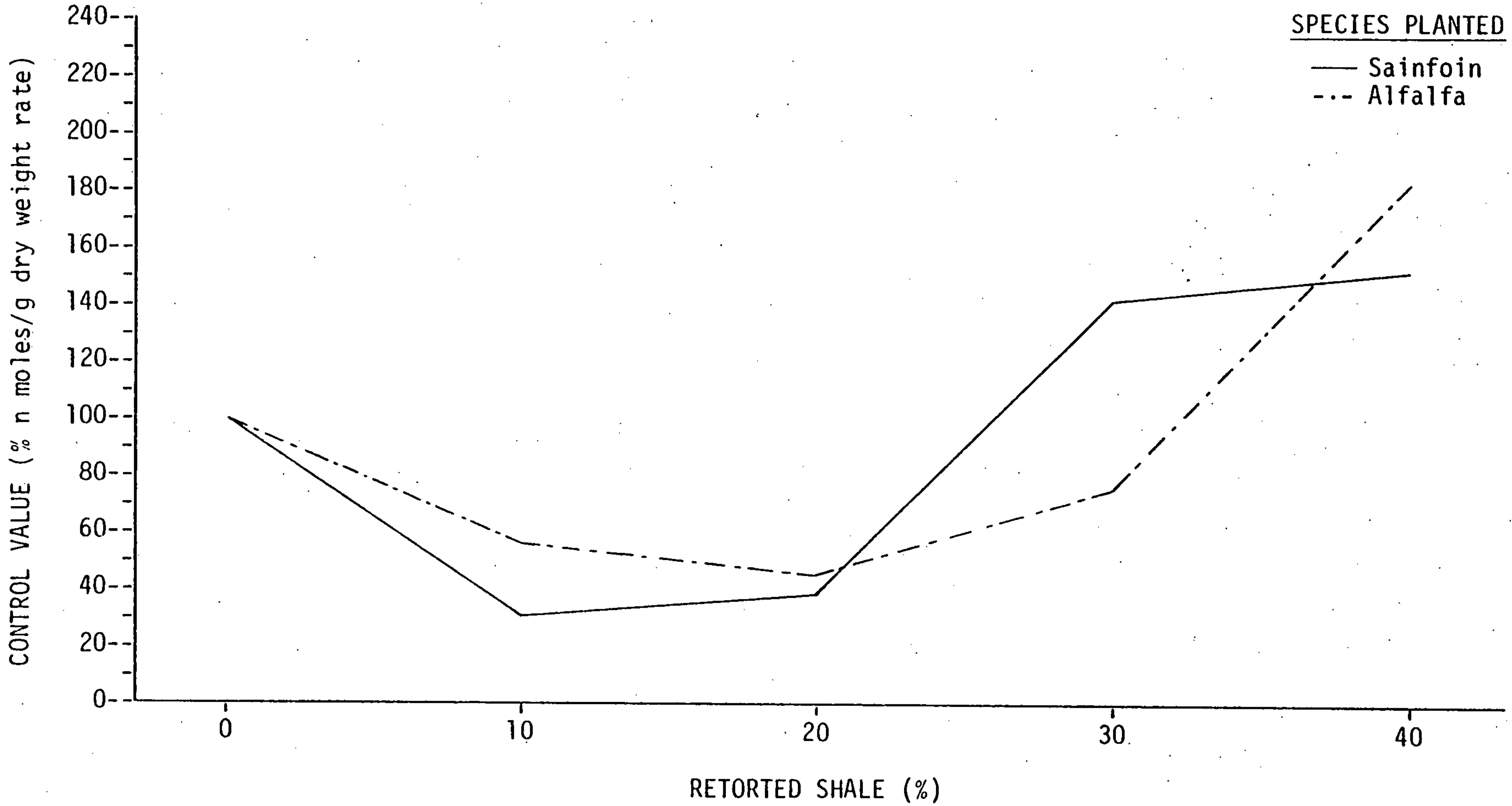

Figure 57. Acetylene reduction by sainfoin and alfalfa planted in soil-retorted shale potting mixtures.

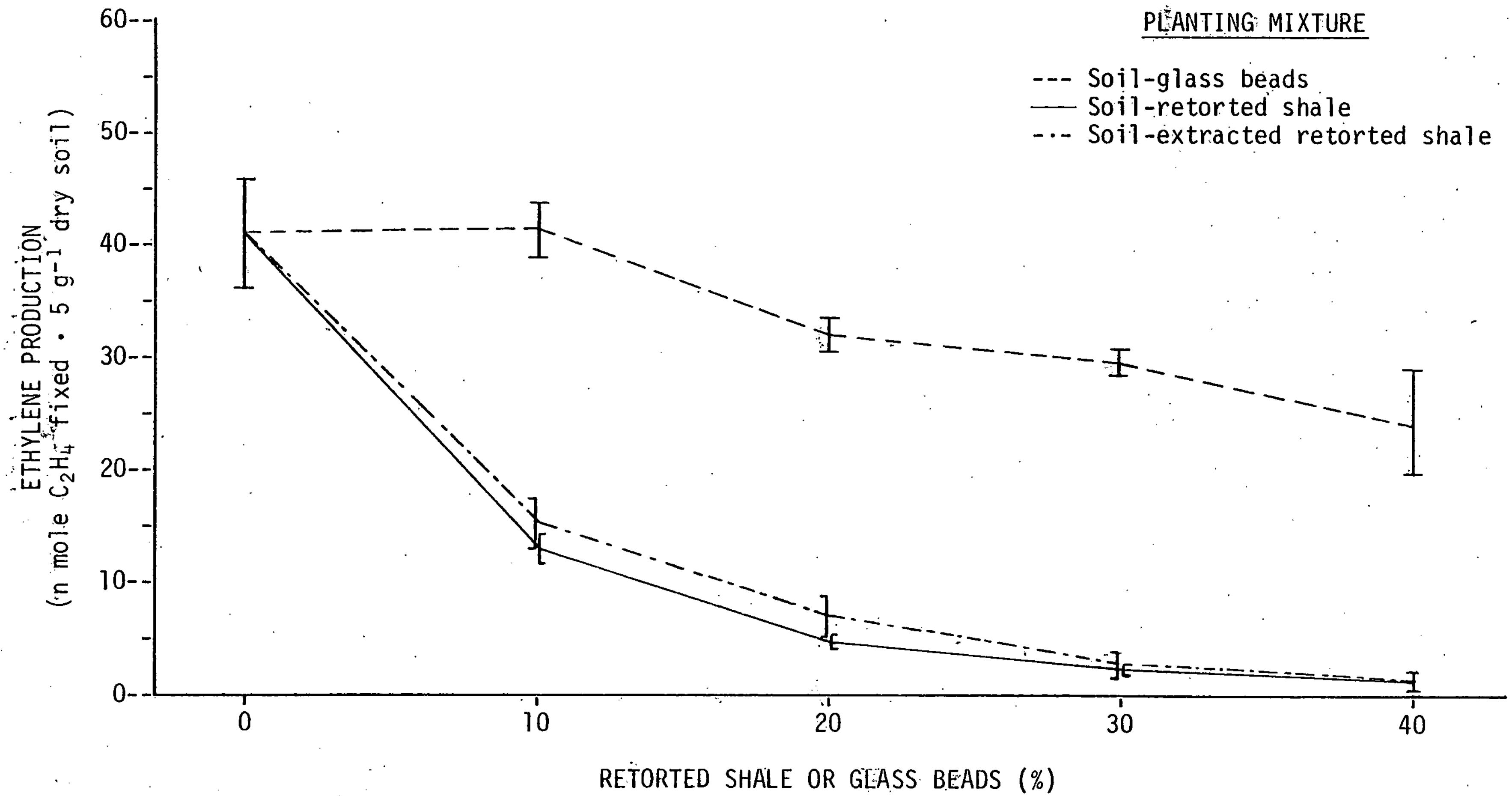

Figure 58. Ethylene production in soil-retorted shale, soil-extracted retorted shale, and soil-glass bead mixtures. (Standard deviations are shown.)

dilution but that there are specific components in the shale which are capable of influencing these processes.

Gassing Mixture Effects on Nodule Nitrogen Fixation Activity

Preliminary results indicate that root nodules show better nitrogen fixation rates with nitrogen than with helium in gassing mixtures. For this reason all root nodule activity assays carried out during this period have used nitrogen-oxygen mixtures, and this procedure will continue to be used in our 1979 field experiments.

Discussion

Revegetation Technique Plot

The management variable which appears to exert the major effect on plant growth and related microbial activity is irrigation. As anticipated, the addition of water to a soil system receiving less than 40 cm of precipitation annualy would induce major responses by the plant-microbe community. The increases in ATP levels, dehydrogenase and phosphatase, nitrogen fixation activity, and percentage organic matter support this concept. Water applications also had long-term effects as irrigation-related changes were noted in the plots which had been irrigated from the previous year.

Equally important, from a revegetation and reclamation viewpoint, were the effects of the other management variables, namely, seed mixtures, fertilizer application, and seeding techniques. All caused direct effects on microbiologically-related parameters. With irrigation the native seed mixture showed the higher levels of accumulated soil organic matter, while without irrigation the introduced species mixture appears to have allowed a more distinct accumulation of soil organic matter. In addition, a trend towards decreased N_2 fixation potential with fertilizer nitrogen additions was noted in comparison with the non-fertilized plots. For the spring sampling this was significant at $P = < .06$.

The percentage soil organic matter also demonstrated strong correlations with pH, phosphatase activity, and N_2 fixation potential, while phosphatase also correlated with pH and N_2 fixation potential for both sampling periods. Although each parameter measures a different aspect of the microbiological compartment, these strong correlations indicate that these have similar responses to the imposed management variables.

Surface Disturbed Successional Plot

For the successional plot, which was sampled only one time during 1978, seed mixtures had the major effect on the accumulation of organic matter in the soils, with the introduced grass mixture and the native grass-forb-shrub mixture being related to the highest soil organic matter levels, both with and without mulching. This response was independent of fertilization treatments.

Seed mixtures were also significantly related to the percentage moisture. Based on inspection of these figures it is apparent that the native grass-forb-shrub mixture was related to perhaps the best combination of organic matter and moisture. Also, this seed mixture yielded the lowest pH values, the best dehydrogenase activity, and better than average values for phosphatase activity and nitrogen fixation potential. Therefore, from a microbiological standpoint this diverse native mixture could be considered to provide the best seeding strategy based on two years of experimentation. These results also suggest that fertilization may not play an essential role in the establishment of these plant-microbe related processes.

Soil Storage Plot

With surface storage of the test soil, the organic matter and percentage moisture decreased while pH demonstrated an inverse relationship with depth. Soil storage studies carried out over a three- to four-month period indicate that without establishment of plant cover soil microbiological activity and soil organic matter levels will decrease more rapidly than when soils are stored under subsurface conditions. This may be due to wetting and drying stimulating microbial activity, which has been shown to lead to decreases in soil organic matter levels (Birch 1958, Powelson 1975).

Plant-Associated Nitrogen Fixation

With increasing concentrations of retorted oil shale above- and belowground plant biomass exhibited a general decrease for both the control and the two leguminous species. Obviously, from a biomass-yield standpoint retorted oil shale provided a less hospitable environment than natural conditions.

The response of root nodules to retorted oil shale is less obvious, though equally as important. Based on our experiments to date, with increasing concentrations of retorted oil shale the number of nodules per gram dry root initially decreased, but beyond a 20 percent retorted oil shale addition level the number of nodules did not change, and for alfalfa an increase was shown. A similar pattern was observed when the

N_2 fixation potential was examined in relation to the added shale levels. After an initial depression (at 10 to 20 percent shale) the nitrogen fixation increased to rates much higher than those shown for control soil with higher levels of retorted oil shale in the soil-shale mixtures.

These observations have distinct management implications, and the possibility of this effect being due to laboratory manipulation and washing techniques is also being evaluated.

Nonplant-Associated Nitrogen Fixation

From our experiments to date it appears that plant-associated and nonplant-associated nitrogen fixation respond differently to the presence of retorted oil shale. Based on the results carried out with sterile glass beads it is apparent that the reduction of N_2 fixation potential in the soil-retorted oil shale mixture reported previously cannot be attributed to a simple dilution effect. No significant reduction in N_2 fixation potential was observed in the glass bead experiment.

Based on the ineffectiveness of methylene chloride to remove the active components in retorted oil shale it can be suggested that the "active" fraction(s) of retorted oil shale is an inorganic substance. What remains to be determined is the identity of the inorganic inhibitor and why nitrogen fixation response patterns differ in plant-associated and nonplant-associated systems.

This information should help us interpret our results for field measurements of legume nitrogen rates which are planned for the 1979-1980 program.

Summary

The results of the 1978 field and laboratory microbiological studies indicate that the management technqiues which are being tested in this program will have distinct effects upon belowground soil microbiological porcesses. Different planting mixtures may be best suited for use under irrigated or nonirrigated conditions, and nitrogen additions did not appear to have major effects on reestablishment of microbiological processes. There is some indication that mineral nitrogen additions may lead to a decreased nitrogen fixation potential. Generally, the addition of retorted shales to surface soils will lead to a decrease in microbiological activities, with a distinct effect on nitrogen fixation by free-living organisms having been observed. Studies with *Rhizobium* nitrogen fixation (both in pure culture and in conjunction with legumes) has shown stimulation and inhibition effects for retorted shales and retorted shale extracts. These studies suggest that mixing of soils with shales and movement of leachable shale components to surface soil zones should be minimized. Topsoil storage studies carried out during 1978 using soils where plant cover had not been established have shown marked decreases in soil organic matter and microbiological activity in surface soils, where temperature and moisture variations are more frequent.

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ABSTRACTS OF PUBLICATIONS OR PRESENTATIONS

First International Rangeland Congress, Denver, Colorado.
14-18 August 1978

TITLE OF PAPER: Seasonal dynamics and responses of rhizosphere and rhizoplane microorganisms associated with semi-arid grassland plants.

ABSTRACT:

To evalute the possible contributions of bacteria, actinomycetes and fungi, as broad gropus, in the rhizosphere and rhizoplane zones of plants in a semi-arid grassland, surface soil cores have been taken at two-week intervals. Respiration (CO_2, O_2), and dehydrogenase activity (DHA) in the presence of five general substrates (glucose, starch, cellulose, amino acids, proteins), viable count estimates of bacteria, actinomycetes and fungi, mycorrhizal and free fungi by microscopic proceudres, and associated biotic and abiotic parameters were measured. Seasonal changes in the contributions of bacteria and fungi to mineralization processes in the rhizosphere and rhizoplane were evaluated by use of a laboratory-scale labelled substrate mineralization assay in which selected antibiotics were used. The dynamics of nitrogen fixation by microbes associated with range grasses were also evaluated. The data were analyzed using a matrix analysis program for the whole year, and for the spring, summer and fall periods, to determine the degree of relatedness (direct and inverse) in changes between the various parameters. Generally, changes in fungal viable counts in the rhizosphere and rhizoplane showed correlations with cahnges in respiration, which were not shown by the bacteria and actinomycetes. The microscopic fungal measurement, in contrast, did show changes related to viable counts for bacteria and actinomycetes. These results suggest that fungal populations in the rhizosphere and rhizoplane may be more sensitive to changes in substrate availability as measured by respiration rate changes (carbon dioxide evolution, oxygen use), than bacteria and actinomycetes, and that fungi may be more responsive to changes in substrate availability in the rhizosphere-rhizoplane microenvironment of semi-arid grassland plants than had previously been considered. Mineralization studies using whole soil fraction demonstrated turnover times of 1-8 hours for glucose and mixtures of amino acids, while starch and cellulose required 2-40 hours respectively for 5% decomposition. During the winter these rates were decreased by approximately 25%. Studies carried out to date suggest that in spite of the relatively large fungal biomass in the decomposer compartment, the bacteria are responsible for the major part of the mineralization processes occurring in the rhizosphere-rhizoplane zone in this grassland ecosystem.

Presented at the symposium "Microcosms in Ecological Research", Augusta, Georgia
8-11 November 1978

Title: "Simulation of Fungal Versus Bacterial Contributions to Plant Root Substrate Mineralization in Soil Microcosms Using a Rapid Radiotracer-Metabolic Inhibitor Technique"

Authors: J. P. Nakas and D. A. Klein 303/491-6947
Department of Microbiology
Colorado State University
Fort Collins, CO 80523

Abstract:

Bacterial and fungal contributions to the mineralization of a series of root exudate-related substrates were monitored using soils from a semi-arid grassland in a microcosm system. In the course of periodic sampling, selective inhibition of bacterial and fungal activities was determined using streptomycin and actidione, with glucose, an equimolar amino acid mixture, starch, cellulose, and ground root materials used as substrates. Microcosms were amended with unlabeled substrates and sampled periodically over a 21-28 day period. Assays for fungal versus bacterial contributions to mineralization potential were carried out using the appropriate ^{14}C-labeled substrate and also ^{14}C-labeled glucose. Based on this assay, bacteria were dominant in the mineralization of the low molecular weight substrates (glucose and the amino acid mixture). However, the fungal component was the major contributor to the mineralization of starch and cellulose when these substrates were used in the final assay, but not when these soil mixtures were assayed using ^{14}C-glucose. Microscopic fungal biomass, phosphatase activity, and ATP levels were also monitored. These date provide additional information on bacterial and fungal contributions to the decomposition of a range of plant-root derived substrates, and suggest that this technique may be useful in monitoring microbial decomposition processes in the rhizosphere-rhizoplane zone under field conditions.

American Society of Agronomy Meeting, Chicago, Illinois, December 1978

Title: Correlation Analysis of Seasonal Changes in Plant Root Associated Fungai-Soluble Inorganic Phosphorus-Pyrophosphatase Relationships in a Semi-arid Grassland Ecosystem

Authors: D. A. Klein*, J. P. Nakas, and N. A. Nagle
Department of Microbiology
Colorado State University
Fort Collins, CO 80523

Abstract:

A correlation analysis procedure was used to evaluate the functioning of mycorrhisze in a semi-arid grassland, using field samples taken at 2-week intervals during 1977. Vesicle presence in the root apical region showed a strong correlation with changes in root biomass and root organic matter content parameters. However, vesicle presence was not related to soil inorganic phosphorus changes. An inverse relationship between vesicle presence and the pH 5.5 pyrophosphatase was noted using frozen soil samples, while no such relationship was noted using unfrozen soil samples, or with pyrophosphatase measurements at pH values of 6.5 or 8.5. These results provide field derived evidence that vesicles do not appear to play a role in phosphorus transfer, but may be more related to the transfer of carbon from the plant to the mycosymbiont. A microscopic fungal biomass showed strong correlations with a chemical assay for glucoseamine, indicating that this chemical assay could be used for minitoring plant-mycorrhizal interactions under field conditions.

18 August 1978

To be presented at the 1979 Society of Range Management Meeting, February 11-14, 1979, Casper, Wyoming

TITLE: Storage Effects on the Microbiological Characteristics of Surface Soils Used in Oil Shale Revegetation Programs

AUTHORS: D. A. Klein, L. E. Hersman and F. B. Reeves, Departments of Microbiology and Botany, Colorado State University, Fort Collins, Colorado 80523

Abstract

The effects of surface versus subsurface soil storage on the microbiological characteristics of a soil planned for use in revegetation over unretorted and retorted oil shale materials were evaluated in this study. In April of 1978, a surface soil storage pile of approximately 2.2 meters depth and 6 x 15 meters width and length, with sloping sides, was installed at the Piceance Creek Revegetation Technique study site. After 3.5 months of storage, without establishment of vegetation, differences in the microbiological characteristics of surface (0-40 cm) and subsurface (80-150 cm) soils were observed. The surface soils showed a decreased potential for supporting nitrogen fixation and the soil from this zone, subject to greater temperature and moisture variations, had an organic matter level approximately 0.1-0.2% lower than that of the soil stored at greater depths. The surface soils also had decreased microbiological populations, phosphatase activities, and exhibited a soil pH approximately 0.1 unit higher than the soils stored under subsurface conditions. By use of a laboratory plant infection assay, the mycorrhizal colonization potential of soils stored under surface and subsurface conditions was found to decrease with time. These results suggest that if soils are to be stored without the establishment of a plant community, storage at greater depths may help to preserve all-over microbial activity, although mycorrhizal infection potential will be decreased. If possible, plants should be established on such materials, to provide an input of organic carbon and to allow maintenance of the mycorrhizal infection potential of these soils.

August 28, 1978

To be presented at the Symposium on Oil Shale Sampling, Analysis and Quality Assurance, to be held 26-28 March, 1979, in Denver, Colorado.

TITLE: Monitoring of Retorted Shale Effects on Non-symbiotic and Symbiotic Nitrogen Fixation Processes: A Factor in the Design and Management of Stored Topsoil Use in Revegetation Programs

AUTHORS: D. A. Klein and L. E. Hersman
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ABSTRACT:

In a laboratory study, the effects of varied additions of retorted oil shale to a topsoil to be used in revegetation over retorted shale were monitored, to determine the relative sensitivity of a series of key microbiological processes to the presence of these materials. Asymbiotic nitrogen fixation was found to be particularly sensitive to the presence of retorted shale, among the parameters tested (Hersman and Klein, submitted for publication). This suggests that the measurement of asymbiotic nitrogen fixation may provide a convenient, inexpensive means of evaluating the potential effects of various oil shale retorting residues on biogeochemical cycling processes in soils. With the sensitivity of nitrogen fixation by free living microorganisms, or possibly by microbes associated with grassland legumes to these materials, the availability of methodology for the evaluation of retorted shale effects on nitrogen fixation would appear to be useful in an oil shale management program.

Am. Soc. Microbiology Meeting - Los Angles, California, May 1979

Title: Phosphatase Activity as an Indicator of Selective Microbial Decomposition in a Semi-arid Grassland.

Authors: J. P. Nakas* and N. J. Nagle.
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Abstract:

Phosphatase activity was monitored at 3 pH levels (5.5, 6.5, 8.5) in soil cores taken from the Pawnee National Grasslands during 1977-78. When assayed in control soils as well as soils amended with 1.0% glucose, starch, or cellulose, the phosphatase activity profile was pH 6.5 > 5.5 > 8.5. However, soils amended with an amino acid mixture demonstrated alkaline phosphatase activity which increased several fold (150-1200 μg PNP released · hr^{-1} · g^{-1} soil) between 18-24 hours. During the same time period, the pH of the soil increased from 6.8 to 336 μg NH_3 - N · g^{-1} soil. In the presence of streptomycin, the alkaline phosphatase activity was inhibited and remained constant for the duration of the experiment. Phosphatase-positive fungi demonstrated an optimum activity at pH 5.5 in 17 of 19 fungal cultures tested, whereas phosphatase-positive bacteria were most active at pH 6.5 and 8.5. Phosphatase assays on axenic cultures of blue grama (*Bouteloua gracilis*) grown in agar and sterile soil, indicated that the dominant grassland plant species contributed only pH 5.5 activity. These data indicate that the use of pH-selective phosphatase activity and possibly other enzyme systems may provide additional information on bacterial and fungal contributions to decomposition processes.

Am. Soc. Microbiology Meeting - Los Angeles, California, May 1979

Title: Effects of a retorted oil shale on non-plant associated and leguminous nitrogen fixation.

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Abstract:

Two experiments were performed to determine the effects of a retorted oil shale, processed by the parahoe procedure, on nitrogen fixation in a western Colorado soil. In the first experiment the retorted oil shale was added to soils in various concentrations. With increasing concentrations of retorted oil shale, significant reduction in non-plant associated nitrogen fixation, as determined by acetylene reduction, was observed. In the second experiment the soil-retorted oil shale mixtures were seeded with two leguminous plants, alfalfa and sanfoin. Although the number of nodules per gram dry plant root was unaffected by an increase in shale concentration, leguminous nitrogen fixation increased with increasing concentrations of the retorted oil shale. These findings suggest that non-plant associated and leguminous nitrogen fixing systems are affected differently by the components in the retorted oil shale sample used in this study.

Am. Soc. Microbiology Meeting - Los Angeles, California, May 1979

Title: Comparison of FITC and FDA Staining with Assays of Physiological Activity in a Soil *Aspergillus* sp.

Authors: E. R. Ingham,* and D. A. Klein
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Abstract:

The relationship between FITC (fluorescein isothiocyanate) or FDA (fluorescein diacetate) staining and physiological activity was examined to evaluate the use of these techniques in the assessment of functional fungal biomass. Batch fermenter cultures of an *Aspergillus* sp. were assayed to assess physiological activity using dry weight, oxygen uptake, phosphatase activity, ATP concentration and 14-C glucose mineralization assays which were compared with fluorescent staining by FITC and FDA. With FITC, hyphae stained uniformly throughout the growth cycle until the onset of autolysis, providing relatively no information on the physiological state of the culture. FDA stained early exponential cells brightly, after which fluorescence decreased, whereas maximum physiological activity as estimated by the other assays occurred later in the growth cycle. These results support the conclusion that FITC is useful in increasing contrast between the cells and background to allow an easier estimation of biomass but further research appears to be required to evaluate the relationship between FDA staining and functional fungal biomass.

Am. Soc. Microbiology Meeting - Los Angeles, California, May 1979

Title: Revegetation Technique Effects on the Microbiological Characteristics of Surface Soil used for Reclamation over Retorted Oil Shale.

Authors: D. A. Klein*, and L. E. Hersman
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Abstract:

The effects of varied planting mixtures (native, introduced, mixed), fertilizer treatments, seeding techniques, and irrigation of the midrobiological characteristics of soil used in revegetation over retorted shale were investigated using test plots located in the Piceance Basin. Over two growing seasons, significant correlations were noted between soil organic matter changes, soil water content, dehydrogenase, phosphatase, N_2 fixation potential and soil ATP, in relation to specific revegetation procedures. For the irrigated sub-plots, maximum organic matter accretion occurred with the native plant mixture, while without irrigation, the introduced plant mixture gave better organic matter increases. These responses were independent of fertilizer treatments, and soils treated with mineral nitrogen tended to have decreased nitrogen fixation potentials, for which effects at $P = < 0.06$ were noted for the spring revegetation technique plot sampling. These results suggest that microbiological process monitoring can be useful in evaluating the more subtle effects of revegetation alternatives for surface soil reclamation after oil shale processing.

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Evaluation of Retorted Oil Shale Effects on the Microbiological Characteristics of Surface Soil Used in Land Reclamation and Revegetation Processes[1]

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(21 manuscript pages)

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ABSTRACT - J. Env. Quality Manuscript

The effects of retorted oil shale additions on the microbiological characteristics of surface soils were investigated in a laboratory study. Soils were mixed with retorted oil shale at 5, 10 and 25% addition levels, and compared with normal soil and retorted shale over a 2.5 month period, with evaluation of microbiological parameters carried out at two week intervals. The effects of shale on these processes were evident within two weeks of incubation, and throughout the remainder of the study similar relative retorted shale effects were noted at the various addition levels used. With retorted shale present at up to 10% by weight, no negative effects on oxygen uptake, or on actinomycete and bacterial viable populations were noted, while significant effects on nitrogen fixation as measured by acetylene reduction, dehydrogenase activity, fungal viable populations, radioactive glucose mineralization, and ATP concentrations occurred. With higher concentrations of retorted shale present, distinct effects on oxygen uptake and actinomycete viable populations also were detectable. These studies suggest that nonsymbiotic nitrogen fixation in surface soils may be especially sensitive to the presence of retorted oil shale. It would appear necessary to assure that sufficient surface soil is used to cover oil shale materials to allow development and functioning of a diverse vegetation-microbiological community; where the influence of underlying shale materials on these processes would be minimized.

Additional index words: retorted-oil shale, environmental effects, surface soils, microbiological characteristics, nitrogen fixation, reclamation processes.

IMPORTANCE OF MYCORRHIZAL FUNGI IN SALVAGING AND STABILIZING DISTURBED SOILS AND RETORTED SHALE WITH NATIVE VEGETATION

Personnel: Dr. F. Brent Reeves, Jr., Associate Professor
Ms. Sue Schwab, Graduate Research Assistant
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OBJECTIVES

The overall objectives of this subproject are to determine the changes in the mycorrhizal fungi following disturbance and to relate these changes to rehabilitation practices. The specific objectives for last year were as follows:

1. To determine the long-term viability of mycorrhizal propagules
2. To extend the survey of the mycorrhizal status of native plants
3. To isolate additional strains of mycorrhizal fungi
4. To determine when revegetation species become mycorrhizal
5. To begin field trials with speices inoculated and noninoculated with mycorrhizal fungi
6. To assess the contribution of mycorrhizae to flowering in grasses
7. To determine the interrelationships of seed mixtures and fertilizers on mycorrhizal potential
8. To analyze mycorrhizal changes in topsoil stored at the Intensive Study Site.

PROGRESS TO DATE

Of our initial eight objectives set for last year seven have been met or are in progress and one was not successful. Our ideas regarding the role of endomycorrhizae in revegetation practices have been formally presented (Reeves et al. 1979). Here we compare an undisturbed and disturbed sagebrush ecosystem and show that there is a strong correlation between the presence of viable propagules of endomycorrhizal fungi and the aboveground vegetation. This concept is extended to primary succession in harsh habitats wherein the absence of mycorrhizal fungi dictates the types of invasion plants which can survive. In this paper we list the mycorrhizal status of the native plants in both disturbed and undisturbed habitats.

In an attempt to more accurately measure the population of viable propagules of mycorrhizal fungi we have formally presented our bioassay (Moorman and Reeves 1979). Under many circumstances the number of spores of these fungi present in the soil is not indicative of the actual potential of the soil to support mycorrhizal formation. In certain circumstances no spores are produced and in other circumstances many of the spores are inviable. Our bioassay measures the active inoculum in the soil without reference to numbers of spores. We have used this bioassay to measure mycorrhizal infection potential (MIP) in variously treated soils at the Intensive Study Site in the Piceance Basin.

Long-Term Viability of Mycorrhizal Propagules

Our first major objective was to determine the long-term viability of propagules of mycorrhizal fungi. Our experimental design uses the

Annual Disturbance Plots. To date our data show that with increasing disturbance the MIP of the soil falls (Table 52) in both the 1976 and the 1977 duplicate plots. With time we expect the MIP of those plots with little or no vegetation (Treatment 3 and 4) to fall whereas those plots in which the vegetation recovers (Treatments 1 and 2) should exhibit increased MIP. In both Treatments 1 and 2, 1976, the vegetation is now recovering from the disturbance and the MIP is higher than the Treatments 1 and 2, 1977, plots where the vegetation has not yet recovered. In contrast, in the severely disturbed Treatment 4 the MIP is less in the 1976 plots than in the 1977 plots. In this case we believe the decrease in the 1976 plots is due to death of the viable mycorrhizal propagules since there is little or no vegetation to support their growth and survival. Additional years' data will confirm these data and indicate the long-term viability under natural conditions (Reeves et al. 1979).

Mycorrhizal Status of Native Plants

Our second objective was to extend the survey of the mycorrhizal status of native plants in the semiarid West. Table 53 and the compilation in the paper by Reeves et al (1979) lists the current status of our survey. Some interesting results have been found to date. Certain traditionally nonmycorrhizal families contain a few species which appear to be mycorrhizal under native conditions, e.g., *Atriplex canescens*, *Ceratoides lanata*, *Eriogonum lonchophyllum*, *E. effusum*, *Rumex crispus*, and *R. venosus*. We intend to carefully study the mycorrhizal infection on *Atriplex* and *Ceratoides* since both these species are important revegetation shrubs for arid sites. Of equal interest is the absence of mycorrhizae on both *Menzelia nuda* and *Oenothera nuttallii*. Both these

Table 52. A comparison of mycorrhizal infection potential (MIP) in soils subjected to increased disturbance (Treatments 1-4) for the Annual Disturbance Plots. (Note the MIP decreases with increased disturbance.)

Treatment Number	1976	1977
1	83.6	67.1
2	81.9	64.9
3	58.6	44.5
4	32.8	45.6

Table 53. Endomycorrhizal associations across a dry grassland creek bed.

Species Planted[1]	Constancy (%)	Number of Plants, Number of Sections (cm)[2]	Occurrence[3]	Degree of Infection[4]
Phylum Pteridophyta				
Family Equisetaceae				
Equistem kansanum	20	3, 5	N,T	M**
Phylum Spermatophyta				
Subclass Monocotyledons				
Family Graminae				
Agropyron smithii	100	6, 60	C,N,T	M**
Bouteloua gracilis	100	3, 30	N	M**
Bromus tectorum	70	3, 30	T	M**
Elymus canadensis	40	3, 30	C,T	M**
Oryzopsis hymenoides	100	6, 60	C,N,T	M**
Stipa commata	20	3, 30	N,T	M**
Subclass Dicotyledons				
Superorder Magnoliales				
Order Papaverales				
Family Papaveraceae				
Argemone platyceras	100	4, 30	C,T	M*
Superorder Caryophyllidae				
Order Caryophyllales				
Family Nyctaginaceae				
Mirabilis hirsuta	30	6, 15	N	M-
Mirabilis linearis	10	1, 25	N	M-
Family Chenopodiaceae				
Atriplex canescens	100	3, 30	N	M**
Chenopodium album	90	4, 60	C,N,T	M-
Chenopodium leptophyllum	50	3, 30	N,T	M-
Corispermum hyssopifolium	60	2, 15	C	M-
Eurota lanata	100	2, 30	N	M*
Kochia scoparia	100	6, 60	C	M-
Monolepis nuttalliana	60	3, 30	C	M-
Salsola kali	100	6, 60	C,N,T	M-
Family Amaranthaceae				
Amaranthus graecizans	50	3, 20	C	M-

Table 53.--Continued

Species Planted[1]	Constancy (%)	Number of Plants, Number of Sections (cm)[2]	Occurrence[3]	Degree of Infection[4]
Order Polygonales				
Family Polygonaceae				
Eriogonum effusum	100	3, 30	N	M*
Polygonum aviculare	10	2, 25	C	M-
Polygonum ramosissimum	80	3, 30	C	M-
Rumex crispus	10	1, 30	T	M***
Rumex venosus	100	5, 55	C,T	M*
Phylum Spermatophyta				
Subclass Dicotylendon				
Superorder Dilleniidae				
Order Malvales				
Family Malvaceae				
Sphaeralecea coccinea	100	3, 30	N	M**
Order Violales				
Family Loasaceae				
Menzelia nuda	70	3, 10	C,T	M-
Order Salicales				
Family Salicaceae				
Populus sargentii	10	No roots were collected		
Salix exiqua	20	1, 30	T	M**
Order Capparales				
Family Capparaceae				
Cleome serrulata	10	1, 30	T	M*
Polanisia trachysperma	100	3, 30	C	M*
Family Cruciferae				
Descurania pinnata	10	Roots too old to sample		
Lepidium densiflorum	20	Roots too old to sample		
Sisymbrium altissimum	30	Roots too old to sample		
Superorder Posidae				
Order Rosales				
Family Saxifragaceae				
Ribes aureum	50	1, 30	N,T	M***
Family Rosaceae				
Rosa acicularis	30	1, 30	N,T	M***

Table 53.--Continued

Species Planted[1]	Constancy (%)	Number of Plants, Number of Sections (cm)[2]	Occurrence[3]	Degree of Infection[4]
Order Fabales				
Family Leguminosae				
Astragalus bisulcatus	20	1, 30	N	M***
Astragalus pectinatus	30	1, 30	N	M**
Glycyrrhiza lepidota	20	3, 30	T	M**
Melilotus alba	40	2, 30	T	M***
Melilotus officinalis	20	2, 30	T	M***
Petalostamen candida	30	2, 30	C	M***
Psoralea lanceolata	100	6, 60	C,N,T	M***
Order Myrtales				
Family Onagraceae				
Oenothera nuttallii	100	3, 30	C	M-
Order Santalales				
Family Santalaceae				
Commandra pallida	30	6, 30	N	M**
Order Euphorbiales				
Family Euphorbiaceae				
Euphorbia strictospora	80	3, 30	C	M**
Superorder Asteridae				
Order Polemoniales				
Family Solanaceae				
Physalis hederaefolia	60	3, 30	T	M**
Phylum Spermatophyta				
Subclass Dicotyledons				
Superorder Asteridae				
Order Polemomiales				
Family Solanaceae				
Solanum triflorum	10	Roots too old to sample		
Family Convolvulaceae				
Convolvulus arvensis	20	3, 30	T	M**
Family Boraginaceae				
Cryptantha jamesii	50	2, 25	C,N,T	M*
Cryptantha minima	40	Roots too old to sample		
Order Gentianales				
Family Asclepiadaceae				
Asclepias specious	10	3, 20	T	M**

Table 53.--Continued

Species Planted[1]	Constancy (%)	Number of Plants, Number of Sections (cm)[2]	Occurrence[3]	Degree of Infection[4]
Order Lamiales				
Family Verbenaceae				
Verbena bracteata	80	3, 30	C	M***
Family Labiatae				
Salvia reflexa	10	1, 30	C	M***
Order Plantaginales				
Family Plantaginaceae				
Plantago purshii	10	3, 30	N	M**
Order Scrophulariales				
Family Scrophulariaceae				
Penstemon spp.	20	1, 20	T	M*
Family Orobanchaceae				
Orobanche ludoviciana	10	3, 7	N	M**
Order Asterales				
Family Compositae				
Artemisia draculculoides	100	3, 30	N	M***
Artemisia filifolius	10	1, 30	N	M*
Artemisia frigida	100	3, 30	N,T	M**
Artemisia ludoviciana	50	3, 30	N	M***
Aster erecoides	10	1, 25	N	M**
Aster tanacetifolius	50	4, 40	N,T	M***
Bahia oppositifolia	10	5, 30	T	M***
Cirsium undulatus	100	6, 30	N,T	M**
Chrysothamnus nauseosus	100	1, 30	N	M**
Franseria discolor	100	3, 30	C,T	M***
Grindelia squarosa	20	1, 30	T	M***
Gutierrezia sarothrae	100	3, 30	C,N,T	M***
Helianthus annus	80	3, 30	T,N	M***
Helianthus petiolaris	100	6, 60	C,N,T	M***
Heterotheca villosa	100	6, 60	C,N,T	M***
Iva xanthifolia	60	3, 30	T	M***
Lactuca scariola	10	Roots too old to sample		
Lygodesmia juncea	100	3, 25	N,T	M*
Senecio spartioides	100	6, 60	C,N,T	M*

Table 53.--Continued

Species Planted[1]	Constancy (%)	Number of Plants, Number of Sections (cm)[2]	Occurrence[3]	Degree of Infection[4]
Thelesperma megabotanicum	20	5, 30	T	M**
Tragopogon dubius	10	1, 30	T	M**
Xanthum italicum	60	3, 30	C,T	M***

[1]The species as listed in this table reflect their taxonomic and evolutionary positions as presented by G. L. Stebbins (1974).

[2]The first number of each set represents the number of plants sampled. The second number indicates the total length of root examined for mycorrhizal infection.

[3]C = the plant was found in the creek bed; N = a species was known to occur in the native or upland areas of the flood plain; T = occurrence in the transitional area between the two habitats.

[4]The amount of mycorrhizal infection is indicated by: M- = no infection; M* = light infection; M** = moderate infection; M*** = heavy infection.

species are found on disturbed sites; perhaps their special adaptations include the loss of mycorrhizal status and this allows tham to invade those sites which have reduced MIP. During this next year we intend to sample the vegetation in the harshest undisturbed sited in the Picenace Basin, e.g., steep south-facing slopes and greasewood bottom lands. Additionally, we have soil collected from several very different ecosystems and intend to bait these soils for the species of mycorrhizal fungi present.

Isolation of Additional Strains

The third objective was to isolate additional strains of mycorrhizal fungi. The rationale for this goal was to secure strains of mycorrhizal fungi already adapted to our semiarid conditions. Currently we have soils from microhabitats of the mid elevation sagebrush community under study. We hope to determine if there are species differences in the mycorrhizal fungi which correlate with the microhabitats and/or the host plants. Although most of the literature suggests that there is little host specificity for mycorrhizal fungi, our experiments suggest the contrary. The presence of mycorrhizae in *Atriplex* and *Ceratoides* in nature and the failure of these genera to become mycorrhizal in our experiments with *Glomus fasciculatus* (Kiel et al 1979) suggests that *G. fasciculatus* is not the normal symbiont for these shrubs. Further, the failure of *Stipa viridula* to exhibit any growth response to *G. fasciculatus* but the increased growth shown by *Agropyron smithii* to this same fungus indicates that the nature of the specificity of other strains of *Glomus* should be examined.

Mycorrhizal Infection of Revegetation Species

The fourth objective of our research was to determine when revegetation species become mycorrhizal. Here our rationale was to see if there was a strong correlation between the success of a species and the ontogeny of the mycorrhizal conditions. We had suspected that the most successful revegetation species may be nonmycorrhizal or become mycorrhizal only late in their development. Our data (Figure 59) show that both *Chrysothamnus* (rabbitbrush) and *Artemisia* (sagebrush) become mycorrhizal early in their development and rapidly approach 100 percent infection. *Agropyron* (western wheatgrass) and *Oryzopsis* (Indian ricegrass) are less heavily infected. *Castilleja, Ceratoides,* and *Atriplex* all remained nonmycorrhizal for the 16-week duration of the greenhouse experiment. These data are definitely tentative; however, they do correlate with some of our field observations. Both *Chrysothamnus* and *Artemisia* are not aggressive invaders in disturbed soils; they have high mycorrhizal infection early in their development. One of the more successful grass species on disturbed soil is *Oryzopsis*; it develops mycorrhizae slowly and remains at a low level of infection. The most successful shrubs on disturbed soils are *Atriplex* and *Ceratoides*; both genera do not require mycorrhizae for at least 16 weeks. It is now essential that we examine field specimens of these genera to determine the correlations between the field condition and the greenhouse experiments.

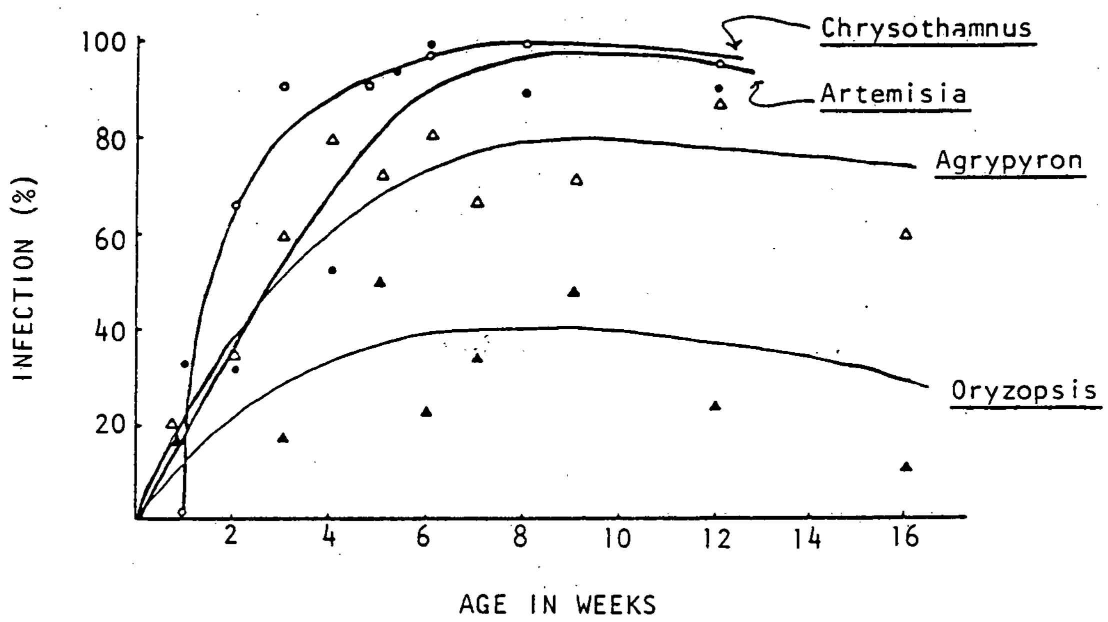

Figure 59.. Ontogeny of mycorrhizal development of native species.

Plant Inoculated Field Trials

We had hoped to begin field trials of artificially inoculated plants during this year, Objective 5. Unfortunately, we have been unsuccessful in establishing vegetative scions of the shrubs. Our visit with the mycorrhizal researchers at the University of Georgia convinced us we had to use the same genetic stock for valid statistical comparisons in field studies. We currently are still trying to establish vegetative cuttings from both sagebrush and rabbitbrush. Our success with the grass species (both *Agropyron* and *Stipa*) will be much greater. Our plans include beginning the grass experiments in the early summer.

Relationship of Mycorrhizae to the Flowering of Grasses

As part of a rather complex experiment involving the effect of soil phosphorus on the growth and development of endomycorrhizae in native plants we observed the effects of mycorrhizae formation in grasses (*Stipa* and *Agropyron*) on flowering, Objective 6. The results of this experiment showed a spectacular increase in the growth of sagebrush (*Artemisia*) when mycorrhizal versus nonmycorrhizal on low P soils (Kiel et al. 1979, abstract). However, neither of the grasses showed increased flowering in the mycorrhizal condition even though *Agropyron* exhibited increased growth in 90 days when mycorrhizal. The results of this experiment will be submitted for publication in the near future.

Seed Mixture and Fertilizer Effects on Mycorrhizal Potential

Our seventh objective was to determine the interrelationships of seed mixtures and fertilizers on mycorrhizal potential in both the

Surface Disturbed Successional Plot and the Revegetation Technique Plot. The results of our data are given in Tables 54 and 55. On the Successional Study Plot there are no significant differences between mulched and unmulched plots or between plots receiving various amounts of fertilizer (phosphorus). However, these plots do show significantly decreased mycorrhizal infection potential (mean of 53.9 percent) when compared with the undistrubed mid elevation sagebrush community (mean of 95.3 percent). These values correlate with the Annual Disturbance Plots (Table 52) where it was found that disturbance leads to decreased mycorrhizal infection potential. It is important that plants in these plots be examined to see if there is a decreased or modified amount of mycorrhizal infection in the successful species. On the more heavily disturbed Revegetation Technique Plot an even greater reduction in the mycorrhizal infection potential than on the Successional Study Plot (38.8 percent as compared to 53.9 percent) was found. Although there are no significant differences between the irrigated versus nonirrigated or fertilized versus nonfertilized subplots studied, we do find a MIP value (38.8 percent) which lies between the values of Treatments 3 and 4 on the Annual Disturbance Plots. Although these values are not absolute fits, there is good correlation between various treatments of the soil and the calculated mycorrhizal infection potential. Again we must determine the degree of mycorrhizal formation in the native plants on these plots in order to obtain data for field comparisons with greenhouse conditions. For these plots some of the most important information remains to be generated. When data is available for success of species and vegetation cover, correlations can be made between our data on the mycorrhizal infection potential of various treatments, soils, and fertilizers.

Table 54. A comparison of mycorrhizal infection potential (MIP) in selected plots (E1, E2, and E3) in the Surface Disturbed Successional Plots. (MES = mean MIP value for undisturbed soil; SSP = mean value for these plots.)

Rep.		Selected Plots E1	E2	E3	
			Mulched		
I		40	66	25	
II		65	53	49	$\bar{x}$ = 52.3
III		48	70	55	
	$\bar{x}$	51	63	43	
			Unmulched		
I		63	49	38	
II		41	56	66	$\bar{x}$ = 55.6
III		53	63	71	
	$\bar{x}$	52	56	58	

MES $\bar{x}$ = 95.3 SSP $\bar{x}$ = 53.9

Table 55. A comparison of mycorrhizal infection potential (MIP) in selected Revegetation Technique Plots (irrigated and nonirrigated) reseeded with native species.

	Irrigated		Nonirrigated	
Nonfertilized	$\bar{x}$ = 48.7	$\bar{x}$ = 42.7	$\bar{x}$ = 33.5	$\bar{x}$ = 34.2
Fertilized	$\bar{x}$ = 36.7		$\bar{x}$ = 34.7	

MES $\bar{x}$ = 95.3 RTP $\bar{x}$ = 38.8

Effect of Topsoil Storage on Mycorrhizal Infection Potential

Our last objective, Objective 8, was to analyze changes in the mycorrhizal infection potential in topsoil during storage. Results from our assay indicate that no substantial reduction in the mycorrhizal infection potential occurs after four months of storage (Figure 60). We have sampled this storage pile at six months, but the data for this experiment is not yet complete. We intend to continue to make careful correlations of our data with that of Dr. Klein in the periodic sampling of this stored soil (Klein et al, 1979, abstract).

Other Investigations

In addition to the eight objectives set for this year we have made some progress in other areas in our attempts to better understand the microbiology of the Piceance Basin. Other investigations not funded by this grant include:

1. A preliminary analysis of the major lichen species in the mid elevation sagebrush community. This work indicates that *Collema tenax*, a crustose lichen containing a blue-green symbiont, has a ground cover of 0.26 percent--well within the range of some of the "major" higher plant species in this community. This lichen may be an important member of the community because of its potential ability to fix atmospheric nitrogen. If possible, we intend to pursue the role of this lichen in the ecosystem.
2. A preliminary analysis of the thermophilic saprobic fungi in the soils of the mid elevation sagebrush community. This work

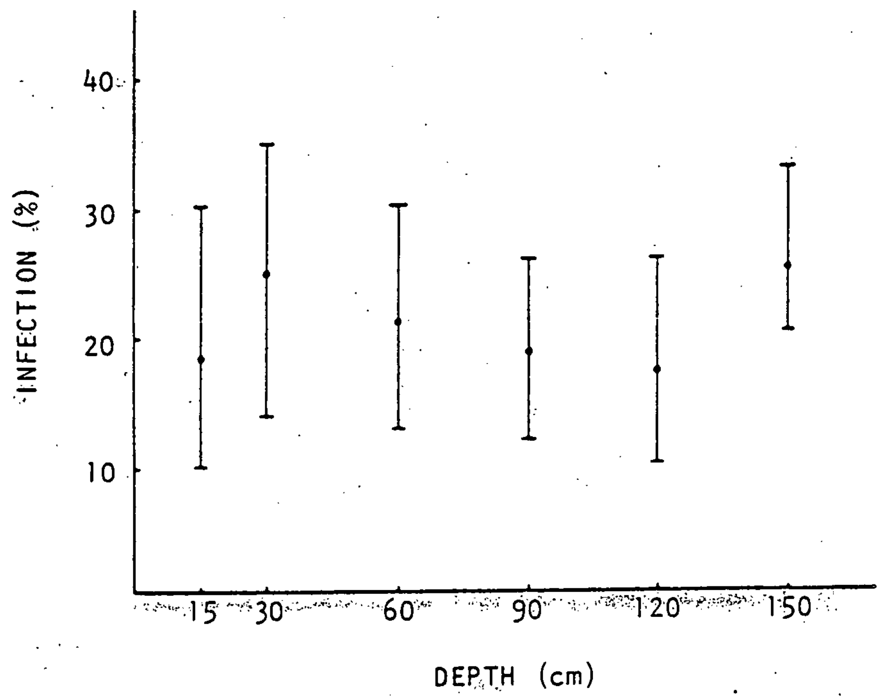

Figure 60. Mycorrhizal infection potential as a function of depth in Topsoil Storage Pile, July 1978.

indicates that a substantial population of thermophils or faculative thermophils live in the soils of this ecosystem. The generic composition of this thermophilic community include most of the reported genera. What their role in the ecosystem may be is not known at this time.

Finally, we have finished our study of the vertical distribution of VA mycorrhizal infection potential in the mid elevation sagebrush community (Schwab and Reeves 1979). This research indicates that below 30 cm depth the mycorrhizal infection potential falls rapidly to zero at less than 1 m depth. This information is important in defining topsoil in terms of its biological activity. Since we know that mycorrhizal infection potential decreases with disturbance, this information is useful in minimizing the decrease due to dilution with soils obtained from depths greater than 30 cm. This research is currently under review for publication.

Summary

In summary we have met most of our objectives, we have begun to measure the fluctuations in mycorrhizal infection potential under field conditions, and we have better defined potential problems and fruitful areas of research. We are now at a stage where various measurements, e.g., aboveground ecosystem success (revegetation success), mycorrhizal infection potential, and various other microbiological processes, can be synthesized into a coherent concept of the interrelationships of the belowground processes to the aboveground ecosystem. Such an understanding is essential for its application to successful revegetation techniques.

LITERATURE CITED

Kiel, J., F. B. Reeves, E. Redente, and C. W. Cook. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. IV. Soil phosphorus effects on growth and endomycorrhizal development in native plants. [To be submitted to the Amer. J. Bot.]

Moorman, T., and F. B. Reeves. 1979. The role of endomycorrhizae in revegetation in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. Amer. J. Bot. 66:14-18.

Reeves, F. B., D. Wagner, T. Moorman, and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. Amer. J. Bot. 66:6-13.

Schwab, S., and F. B. Reeves. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. III. Vertical distribution of VA mycorrhizal infection potential. [Submitted to the Amer. J. Bot. in December 1978, currently under review.]

ABSTRACTS OF PUBLICATIONS OR PRESENTATIONS

F. Brent Reeves, Department of Botany and Plant Pathology, Colorado State University, Fort Collins.

THE ROLE OF ENDOMYCORRHIZAL FUNGI IN RECLAIMING DISTURBED OIL SHALE LANDS

In a native, mid-elevation sage brush community (MES) located near the focus of oil shale activity in Western Colorado 100% of the 12 major species of higher plants are infected with vesicular-arbuscular mycorrhizal fungi. In sharp contrast, in an adjacent heavily disturbed site (DS), the vegetation is totally different, consisting primarily of weedy species; less than 1% of these species are infected with mycorrhizal fungi. A bioassay using corn confirmed the great reduction of viable propagules of the mycorrhizal fungi in the disturbed area. When grown in MES soil the corn roots were 77% infected; when grown in DS soil the corn roots were 2% infected. The strong correlation between stable, sage ecosystems and abundant mycorrhizal fungi and the absence of these fungi in disturbed areas indicates that maintaining or reestablishing these essentail fungi is necessary when attempting to rehabilitate disturbed soils to a stable ecosystem.

15 min   2 x 2 slides

To be presented at the EPA Oil Shale Sampling, Analysis and Quality Assurance Symposium, Denver Research Center, Denver, Colorado. March, 1979.

REEVES, F. BRENT, T. MOORMAN, S. SCHWAB, AND E. REDENTE. Correlations Among Land Disturbance, VA-Myocrrhizal Infection Potential, and Vegetation Cover in a Mid-Elevation Sage Community.

In 1976 duplicate 6 x 8 m Annual Disturbance Plots, consisting of four increasingly harsh disturbance treatments, were established in a mid-elevation sage (MES) community. In Treatment 1 the vegetation was scrapped with a caterpillar blade; in Treatment 2 the vegetation was stripped and the soil ripped to 30 cm; in Treatment 3 the topsoil and subsoil were mixed to a depth of 1 m; and in Treatment 4 two m of soil were removed and replaced in reverse order. None of the plots were reseeded. Using a bioassay the mycorrhizal infection potential (MIP) of the undisturbed MES community and the four Treatments were compared. The MES had an MIP of 95.3%. Treatments 1, 2, 3, and 4 had MIP's of 83.6, 81.9, 58.6, and 32.8 %, respectively. Vegetation cover for the MES was 22.4 %. Treatments 1-4 had vegetation covers of 10.05, 0.8, 0.05, and 0.0 %, respectively. Similar plots established in 1977 showed similar results except that the MIP of Treatment 4 was significantly higher than in the 1976 plots. We conclude from these data that MIP is a useful measure of the natural rehabilitation or revegetation potential of disturbed soils in the semi-arid West.

April 1979
PAPER PROPOSED FOR THE Botanical SCIENCES SECTION
SOUTHWESTERN AND ROCKY MOUNTAIN DIVISION
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE
COLORADO-WYOMING ACADEMY OF SCIENCE
Fort Lewis College, Durango, Colorado 81301

Author(s): F. Brent Reeves, T. Moorman, S. Schwab, and E. Redente
(If paper is of joint authorship, underline the name of the one who will make the presentation at the meetings.)

INSTITUTIONAL CONNECTION OF AUTHOR(S): Department of Botany and Plant Pathology and Department of Range Science, Colorado State University, Fort Collins

PREFERRED DATE FOR PRESENTATION: (If you will be available to present the paper only within certain time limits, please specify.)

TIME REQUIRED FOR PRESENTATION: 15 min

PROJECTION EQUIPMENT NEEDED:
(only 2" x 2" can be assured)

TITLE OF PAPER: Correlations among land disturbance, VA-mycorrhizal infection potential, and vegetation cover in a mid-elevation sage community.

☐ Indicate here if this is a student paper to be considered for an award.

Please remove this sheet and attach to copy of Abstract.

BISHOP, CLINTON L. Effects of Light Intensity on Vesicular-Arbuscular Mycorrhizal Infection in *Zea mays*.

Corn plants were cultivated over a 24 day period in a growth chamber (14 hrs light at 30°C and 10 hrs dark at 20°C) under three different light intensities. Plants grown under 400 ft-c showed significantly lower levels of vesicular-arbuscular endomycorrhizal infection and less plant growth enhancement as compared to plants grown under the higher light intensities (800 and 1600 ft-c). These results demonstrate the importance of controlling environmental factors when evaluating the effects of endomycorrhizal fungi on growth enhancement of higher plants.

April 1979
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AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE
COLORADO-WYOMING ACADEMY OF SCIENCE
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Colorado State University, Fort Collins

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TIME REQUIRED FOR PRESENTATION: 15 min

PROJECTION EQUIPMENT NEEDED:
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TITLE OF PAPER: Effects of Light Intensity on Vesicular-Arbuscular Mycorrhizal Infection in *Zea mays*

☒ Indicate here if this is a student paper to be considered for an award.

Please remove this sheet and attach to copy of Abstract.

SCHWAB, SUZANNE. Rate of Formation of VA Mycorrhizae in Seedlings of Seven Species Native to the Mid-Elevation Sage Community of Northwestern Colorado.

Seedlings of seven plants native to the mid-elevation sage community of the Piceance Basin in northwestern Colorado were grown in the greenhouse in soil collected from an undisturbed site in the Piceance Basin and sampled at weekly intervals to determine the age at which these plants became mycorrhizal, and the length of time required for each species to reach its maximum level of infection. Artemisia tridentata, Chrysothamnus nauseosus, and Agropyron smithii became heavily infected within four weeks of seedling emergence and maintained these high levels of infection throughout the four month sampling period. Sphaeralcea munroana also became heavily infected within four weeks, but the proportion of mycorrhizal roots then decreased as the thick tap root typical of this species developed. Oryzopsis hymenoides became only moderately mycorrhizal and showed the greatest variability in regard to mycorrhizal formation among the species examined. Neither Atriplex canescens nor Ceratoides lanata showed any sign of mycorrhizal formation in six months. These results correlate with the relative success of these species as invaders of disturbed sites, with those species that are least mycorrhizal as seedlings being the most successful invaders.

April 1979
PAPER PROPOSED FOR THE Botanical SCIENCES SECTION
SOUTHWESTERN AND ROCKY MOUNTAIN DIVISION
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE
COLORADO-WYOMING ACADEMY OF SCIENCE
Fort Lewis College, Durango, Colorado 81301

Author(s): Suzanne Schwab
(If paper is of joint authorship, underline the name of the one who will make the presentation at the meetings.)

INSTITUTIONAL CONNECTION OF AUTHOR(S): Department of Botany and Plant Pathology
Colorado State University, Fort Collins

PREFERRED DATE FOR PRESENTATION: (If you will be available to present the paper only within certain time limits, please specify.)

TIME REQUIRED FOR PRESENTATION: 15 min.

PROJECTION EQUIPMENT NEEDED:
(only 2" x 2" can be assured)

TITLE OF PAPER: Rate of Formation of VA Mycorrhizae in Seedlings of Seven Species Native to the Mid-Elevation Sage Community of Northwestern Colorado.

☒ Indicate here if this is a student paper to be considered for an award.

Please remove this sheet and attach to copy of Abstract.

The Role of Endomycorrhizae in Revegetation Practices in the Semi-Arid West. III. Vertical Distribution of VA Mycorrhizal Infection Potential[1]

Suzanne Schwab and F. Brent Reeves[2]
Department of Botany and Plant Pathology
Colorado State University
Fort Collins, Colorado 80523

ABSTRACT

The changes in VA mycorrhizal infection potentials in soil profiles from a mid-elevation sage community were measured using a corn bioassay. VAM infection potential was significantly reduced below 30 cm depth and approached zero at less than 1 m depth. The decrease in infection potential with depth in diluted soils did not always parallel changes in the undiluted soils, indicating factors other than numbers of inoculum units also may be important in determining the extent of mycorrhizal formation. The relationship of these results to land disturbance and associated dilution or reduction of populations of mycorrhizal fungi and to defining topsoil is discussed.

Submitted to
American Journal of Botany
December 1978

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[1]Received for publication ________________.

[2]This work was supported by ERDA Contract EY-76-S-02-4018

To be submitted to
American Journal of Botany
by June 1979

The Role of Endomycorrhizae in Revegetation Practices in the Semi-Arid West. IV. Soil Phosphorus Effects on Growth and Endomycorrhizal Development in Native Plants[1]

Jean E. Kiel, F. Brent Reeves, E. Redente, and C. W. Cook[2]

Department of Botany and Plant Pathology and

Department of Range Science

Colorado State University

Fort Collins, Colorado 80523

ABSTRACT

Mycorrhizal associations with 5 species (*Atriplex canescens*, *Ceratoides lanata*, *Artemisia tridentata*, *Stipa viridula*, and *Agropyron smithii*) were evaluated in terms of their impact on plant growth in the greenhouse. Three soil phosphorus (P) regimes (no P, 25 ppm P, and 50 ppm P) were imposed upon each species. One-half of the plants in each regime were inoculated with the VA mycorrhizal fungus *Glomus fasciculatus*. *A. canescens* and *C. lanata* did not become mycorrhizal. Inhibition of mycorrhizal infection accompanied phosphate fertilization in the other 3 species. Mycorrhizal (M+) treatments of *S. viridula* exhibited no significant increase in growth over the non-mycorrhizal (M-) treatments. At 90 days the *A. smithii* M+ no P treatment exhibited a 35% increase in weight over its non-mycorrhizal counterpart. This effect was not detected after 180 days. The amount of P present in the dried tissue of the grasses did not correlate with the presence of mycorrhizae. The M+ no P treatment of *A. tridentata* exhibited increases of 143% in weight, 81% in height, and 51% in plant tissue P compared with the M- no P treatment. In this species the presence of mycorrhizae accompanied by an increase in P uptake corresponds to increased plant growth. Added P can obviate the need for mycorrhizal fungi under greenhouse conditions.

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[1]Received for publication ________

[2]This work was supported by ERDA Contract EY-76-S-02-4018 and is based on a thesis submitted by JEK.

STORAGE EFFECTS ON THE MICROBIOLOGICAL CHARACTERISTICS OF SURFACE SOILS USED IN OIL SHALE REVEGETATION PROGRAMS

D. A. KLEIN*, L. E. HERSMAN and F. B. REEVES, Departments of Microbiology and Botany—Colorado State University, Fort Collins, Colorado 80523

The effects of surface versus subsurface soil storage on the microbiological characteristics of a soil planned for use in revegetation over unretorted and retorted oil shale materials were evaluated in this study. In April of 1978, a surface soil storage pile of approximately 2.2 meters depth and 6 x 15 meters width and length, with sloping sides, was installed at the Piceance Creek Revegetation Technique study site. After 3.5 months of storage without establishment of vegetation, some significant differences in the microbiological characteristics of surface (0-40 cm) and subsurface (80-150 cm) soils were observed. The surface soils showed a significant decrease in asymbiotic nitrogen fixation potential, and the soil from this zone, subject to greater temperature and moisture variations, had an organic matter level approximately 0.1-0.2% lower than that of the soil stored at greater depths, suggesting that the periodic wetting and drying which can occur in this zone had made the soil organic matter more available for microbiological decomposition. The surface soils also tended to have decreased microbiological populations and phosphatase activities, and exhibited a pH approximately 0.1 unit higher than the soils stored under sub-surface conditions. In contrast, the dehydrogenase activity, with and without glucose amendments, and the mycorrhizal infection potential showed no distinct changes with depth of soil storage over this period. By use of a laboratory plant infection assay, the

mycorrhizal colonization potential of the stored soil was found to range from 10-33% root infection, with an average of 21.08% for all test core and depth replications (SD = 7.55). Undisturbed surface soil (0-15 cm) in this area normally have an infection potential in the range of 34-37%, and the lower infection level observed for the mixed soil from the storage pile was within the range of dilution which would occur in mixing material from the 0-38 cm depth used in construction of the pile. These results suggest that if soils are to be stored without the establishment of a plant community, that storage at greater depths may help to maintain more sensitive microbial parameters, especially asymbiotic nitrogen fixation and minimize organic matter loss through wetting and drying processes. If possible, plants should be established on such materials, to provide an input of organic carbon and to maintain or possibly improve the mycorrhizal infection potential of these soils prior to use in revegetation programs.

Presented at

Society for Range Management Annual Meeting
Casper, Wyoming
12-15 February 1979

Fourth North American Conference On Mycorrhizae

COLORADO STATE UNIVERSITY
Fort Collins, Colorado

24-27 June 1979

CALL FOR PAPERS

I. I would like the following paper(s) to be presented at the subject Conference:

Title	Author(s)	Type of Presentation (Oral, Poster, Abstract Only)	Person to Present Paper
Correlations of VA mycorrhizal fungi and semi-arid ecosystem recovery	F. Brent Reeves	Oral	Reeves

II. Contact address: Botany and Plant Pathology Department, Colorado State University, Fort Collins 80523

III. Please forward to:

Dr. C. P. P. Reid
Department of Forest and Wood Sciences
Colorado State University
Fort Collins, Colorado 80523

by March 1, 1979.

Fourth North American Conference On Mycorrhizae

COLORADO STATE UNIVERSITY
Fort Collins, Colorado

24-27 June 1979

CALL FOR PAPERS

I. I would like the following paper(s) to be presented at the subject Conference:

Title	Author(s)	Type of Presentation (Oral, Poster, Abstract Only)	Person to Present Paper
Effect of Retorted Oil Shale Wastes on VA Mycorrhizal Formation in Soil from Northwestern Colorado	Suzanne Schwab	Oral	Schwab
Effect of Inoculum Density on VA Mycorrhizal Formation and Growth Enhancement in *Artemesia tridentata*	Suzanne Schwab	Oral	Schwab

II. Contact address: Suzanne Schwab
Dept. of Botany and Plant Pathology, CSU
Fort Collins, Colorado 80523

III. Please forward to:

Dr. C. P. P. Reid
Department of Forest and Wood Sciences
Colorado State University
Fort Collins, Colorado 80523

by March 1, 1979.

F. BRENT REEVES*, T. MOORMAN, S. SCHWAB, AND E. REDENTE

Departments of
Botany and Plant Pathology
Range Science

EXPERIMENTAL EVIDENCE FOR THE RECOVERY OF A SEMI-ARID ECOTYSEM AS A FUNCTION OF MYCORRHIZAL INFECTION POTENTIAL OF THE SOIL

Recent evidence suggests substantial reductions in the populations of VA-mycorrhizal fungi following severe disturbance of semi-arid soils. We have suggested that the slow rate of recovery of native vegetation can be related to mycorrhizal infection potential (MIP) of the soil. To test this hypothesis duplicate sets of 6x8 m plots consisting of four increasingly harsh disturbances (1-4) were established in a sagebrush community 1976 and 1977. Our bioassay of the MIP of the undisturbed sagebrush community was 95.3% in 1978. MIP values for the four treatments in the 1976 plots were 83.6, 81.9, 58.6, and 32.8%, respectively, for plots 1-4 in 1978. Vegetation cover values were 10.0, 0.8, 0.05, and 0.0%, respectively, for plots 1-4. The 1977 duplicate plots showed similar results. These data are consistent with our hypothesis that in semi-arid environments natural recovery of ecosystems is correlated with the MIP of the soil.

To be presented at the

BOTANICAL SOCIETY OF AMERICA 7TH ANNUAL MEETING
Oklahoma State University, Stillwater

12-17 August 1979

Amer. J. Bot. 66(1): 6–13. 1979.

THE ROLE OF ENDOMYCORRHIZAE IN REVEGETATION PRACTICES IN THE SEMI-ARID WEST. I. A COMPARISON OF INCIDENCE OF MYCORRHIZAE IN SEVERELY DISTURBED VS. NATURAL ENVIRONMENTS[1]

F. BRENT REEVES,[2] DAVID WAGNER, THOMAS MOORMAN, AND JEAN KIEL

Department of Botany and Plant Pathology, Colorado State University, Fort Collins 80523

ABSTRACT

A comparison of a natural, undisturbed ecosystem, a mid-elevation sage community, with a severely disturbed old roadbed through this community revealed that more than 99% of the plant cover in the natural community was mycorrhizal (vesicular-arbuscular), whereas less than 1% of the plant cover in the disturbed area (roadbed) was mycorrhizal. Examples of nonmycorrhizal plants as primary successional species in severely disturbed habitats are discussed. The importance of maintaining or re-establishing the mycorrhizal fungal component in reclamation programs designed to produce stable ecosystems is emphasized.

A COMBINATION of intractable factors viz., fossil fuel development and fragile ecosystems, necessitate the development of the best methods for re-establishing diverse, stable, and functional plant communities on disturbed land. These ecosystems should be established with a minimum of investment of scarce resources, e.g., fertilizers, water, fossil fuels, and man power. The reestablishment of functional ecosystems presumes a knowledge of both the important macro- and micro-components of the system, i.e., both the above- and below-ground elements constituting the system. Vesicular-arbuscular (VA) mycorrhizae are among the ubituitous components in below-ground ecosystems (Wilhelm, 1966; Gerdemann, 1968, 1971, 1975; Smith, 1974; Read, Koucheki, and Hodgson, 1976); the fungal symbionts appear to be essential in most ecosystems (Hacskaylo, 1972; Mosse, 1973; Gerdemann, 1975); these fungi therefore must be studied to understand ecosystem changes.

As part of our integrated studies on rehabilitation potentials of stable ecosystems in semiarid Colorado oil-shale lands we have been investigating mycorrhizae in native and disturbed habitats. This study specifically compares the incidence of VA mycorrhizal plants in an undisturbed sagebrush community with the incidence of VA mycorrhizal plants in a severely disturbed area adjacent to the undisturbed site.

[1] Received for publication 5 April 1978; revision accepted 12 September 1978.

[2] This work was supported by ERDA Contract E(11-1)-4018 to FBR.

MATERIALS AND METHODS—The undisturbed study site is located near the focal point of oil shale activity in the Piceance Basin in western Colorado. The site is at an elevation of approximately 2,040 m, precipitation is approximately 38 cm annually, and the ecosystem is characterized as a mid-elevation sage community (Ward, Slauson, and Dix, 1974; Sims, 1977) with widely scattered patches of pinyon-juniper associations. Soils are clayey loams or loamy clays up to 160 cm deep over shale.

The disturbed study site is an old roadbed which runs through the sage community. This roadbed was abandoned and then ripped to a depth of approximately 46 cm in 1973. No reseeding of the roadbed was attempted.

The official location of the study sites is as follows: W ½, Sec. 4, T 2S, R. 98 W.

Ten 30 m line intercept transects and five m^2 quadrats along each transect were used to determine the percent cover and frequence of occurrence of species present in the undisturbed community (Sims, 1977). Similarly, in the disturbed old roadbed (approximately 12 m wide), line intercept, quadrat methods were used.

Throughout the spring and summer of 1976 and 1977 at least three specimens of each of the major species present in each area were carefully dug up and the roots were fixed in FAA. In the laboratory the fixed roots were cut into 1 cm sections, cleared in hot 10% KOH, and stained in trypan-blue lacto-phenol (Phillips and Hayman, 1970). The stained sections were mounted in colorless lacto-phenol and examined at 125× and 500× for the presence or absence of mycorrhizal infections. A root was considered infected if it showed hyphae + vesicles, or hyphae + arbuscules, or hyphae + pelotons, or any combination of these structures characteristic of VA infections.

RESULTS—Table 1 lists the species found in each of the sites and the mycorrhizal status of each species. In the mid-elevation sage (MES) community only two of the 42 species present were nonmycorrhizal, whereas on the disturbed site (DS) 15 of the 21 species present were nonmycorrhizal.

A more accurate concept of the relative importance of each species is given in Table 2 in which the mean percent ground cover and mycorrhizal status of each important species is given. In the MES community only 12 species have a percent ground cover greater than 0.1% and *all* these species are mycorrhizal. The antithesis of this condition occurs in the disturbed site: only one species had a percent ground cover of 0.1% or more and this species is nonmycorrhizal.

Soil analyses indicate that there are few differences in the soil from the adjacent sites in spite of the large differences in species composition and mycorrhizal status (Moorman and Reeves, 1979).

DISCUSSION—Abundant research indicates that most plants, both fossil and extant, growing in natural environments are dual organisms—the higher plant root and fungus form a mycorrhiza (Gerdemann, 1968, 1971, 1975; Pirozynski and Mallock, 1975). The interdependence of the plant-fungus relationship has been described as "the ultimate in reciprocal parasitism" (Hacskaylo, 1972). Perhaps because of the apparent ubiquity of mycorrhizal fungi but also because of a general lack of appreciation of these organisms, almost all ecological studies of plant succession have totally ignored these essential mycobionts of the below-ground ecosystem, e.g., Benchmark Papers in Ecology-Ecological Succession has but a single case of the inhibition of mycorrhizal fungi and their role in subsequent succession (Golley, 1977).

In view of the essential nature of mycorrhizal fungi for most plants in native environments it is surprising that a closer examination of the role of the fungi in primary and secondary succession is disturbed habitats has not been carefully examined. Pirozynski and Malloch (1975) suggest that "it may not be merely a matter of coincidence" that a disproportionate number of species that are efficient colonizers of disturbed habitats are in traditionally nonmycorrhizal families (Gerdemann, 1968), although they do not cite examples. Our results provice data which support the hypothesis that nonmycorrhizal plants are effective colonizers of disturbed habitats and that the lack of mycorrhizal fungi exert profound influences on species composition. In our study 99% of the plant cover adjacent to the disturbed habit was mycorrhizal—less than 1% of the plant cover was mycorrhizal in a narrow disturbed area (old roadbed—DS site) surrounded by the mycorrhizal area (MES).

Additional support for the efficiency of nonmycorrhizal plants being colonizers of disturbed areas can be obtained from inferences on research reports for the other disturbed areas. Sims and Redente (1974) concluded that natural revegetation of disturbed surface soils in the semi-arid West is a slow process and the greater the severity of the disturbance the slower the rate of recovery. These conclusions were based on analyses of relatively minor surface disturbances such as construction of roads, pipelines, drill sites, or where topsoil is used to cover overburdened or processed (spent) oil shale material. We suggest that a possible explanation for the delays in recovery is in part due to the elimination or reduction in the number of viable propagules of mycorrhizal fungi (either spores, hyphae, or infected root fragments). Without mycorrhizae the seedlings cannot survive or their growth potential is significantly reduced. Recovery of an ecosystem in part is dependent on either the rate of invasion of the site by propagules of mycorrhizal fungi which are viable or roots having or tolerating mycorrhizal fungi.

Further evidence for our hypothesis is found in the Terwilliger, Cook, and Sims' (1974) study of oil shale ecosystems and their natural and artificial rehabilitation. They reported (in percent composition per plot studied) that vegetation succession following disturbance was as given in Table 3.

An examination of the most prominent species in each ecosystem except the reseeded Bottomland sagebrush community reveals that, after one year, these species are *nonmycorrhizal* (indicated by a double asterisk). In those cases where no reseeding was done almost all the species are nonmycorrhizal.

In the compilation of studies on Processed [Oil] Shale Revegetation Studies, 1965–1973, for Colony Development Operation, data indicate that processed shale (TOSCO II type) can support plant growth if the shale is leached, fertilized with sufficient nitrogen and phosphorus, and the seedlings are subsequently maintained with water and fertilizer (Block and Kilburn, 1973). The long-term result of seedling and transplant experiments on processed oil shale plots cannot be predicted. Many native species fail to survive when planted on shale, and on the experimental plots exotic invaders are among the pioneers when the plots are abandoned to natural condi-

TABLE 1. *Species present at both sites and their mycorrhizal status*

Undisturbed site (MES)		Disturbed site (DS)	
Chenopodiaceae		Chenopodiaceae	
Ceratoides lanata	M+	*Atriplex argentea*	M−
Sarcobatus vermiculatus	M−	*A. confertifolia*	M−
		A. rosea	M−
		Chenopodium album	M−
		C. leptophyllum	M−
		Sarcobatus vermiculatus	M−
		Halogeton glomeratus	M−
		Salsola kali	M−
		Kochia scoparia	M−
Brassicaceae		Brassicaceae	
Physaria floribunda	M−	*Lepidium perfoliatum*	M−
		Physaria acutifolia	M−
		Chorispora tenella	M−
		Descurania pinnata	M−
Polygonaceae		Polygonaceae	
Eriogonum effusum	M+	*Eriogonum lonchophyllum*	M+
E. lonchophyllum	M+	*E. cernuum*	M−
		Polygonum aviculare	M−
Asteraceae		Asteraceae	
Agoseris glauca	M+	*Chaenactus douglasii*	M+
		Chrysothamnus nauseous	M+
Artemisia frigida	M+		
Antennaria rosea	M+		
Aster rubrotinctus	M+		
Chaenactus douglasii	M+		
Chrysothamnus naseousus	M+		
C. vicidiflorus	M+		
Erigeron sp.	M+		
Gutierrezia sarothrae	M+		
Haplopappus acaulis	M+		
H. nuttallii	M+		
Tetradymia canescens	M+		
Townsendia incana	M+		
Malvaceae		Malvaceae	
Sphaeralcea coccinea	M+	*Sphaeralcea coccinea*	M+
Polemoniaceae		Polemoniaceae	
Ipomopsis congesta	M+	*Ipomopsis congesta*	M+
Phlox hoodii	M+		
Leguminosae			
Astragalus diversifolius	M+		
A. purshii	M−		
Hedysarum boreale	M+		
Trifolium gymnocarpon	M+		
Rosaceae			
Purshia tridentata	M−		
Scrophulariaceae			
Castilleja chromosa	M+		
Penstemon sp.	M+		
Euphorbiaceae			
Euphorbia robusta	M+		
raginaceae			
ryptantha sericea	M+		
aceae			
untia polyacantha	M−		
ae		Poaceae	
pyron inerme	M−	*Bromus tectorum*	M−
ithii	M+		
chycaulum	M−		
ua gracilis	M−		
tectorum	M+		

TABLE 1. *Continued*

Undisturbed site (MES)		Disturbed site (DS)
Koeleria cristata	M+	
Oryzopsis hymenoides	M+	
Sitanion hystrix	M+	
Stipa comata	M+	
Pinaceae		
Juniperus osteosperma	M+	
Pinus edulis	M+	

tions. On the "box plots" of shale 60% of the invaders (1972–1973) are members of nonmycorrhizal families. The invaders are not the nonmycorrhizal *Salsola kali*, *Chenopodium album*, *Polygonum aviculare*, and *Sarcobatus vermiculatus* found by Terwilliger et al. (1974) but rather presumably nonmycorrhizal species of *Amaranthus*, *Eriogonum*, *Sisymbrium*, *Chenopodium*, and unidentified crucifers. These studies suggest that the mycorrhizal fungi were eliminated or reduced to the point where they would no longer infect the native, typically mycorrhizal seedlings which germinate in the non-reseeded areas. The reseeding of wheatgrass in certain cases probably maintains a certain percentage of selected mycorrhizal species. However, a monoculture of wheatgrass, because this species may offer a limited host range for the mycorrhizal fungi present in the "topsoil," may limit the numbers of higher plant species which can rapidly replace the grass to those which have the identical mycorrhizal species requirement. Our suggestion that propagules of VA mycorrhizal fungi may be significantly reduced in numbers over short periods of time differs with Gerdemann's (1971) report that VA mycorrhizal propagules can remain viable up to six and one-half years in Illinois soil. However, the extremely dry Colorado soils and the relatively harsh conditions of the oil shale region contrast sharply with the relatively constant conditions of the Illinois soil examined by Dr. Gerdemann. Moorman and Reeves (1978) have demonstrated that percent infection in roots is a function of numbers of viable propagules present. Extrapolation of their data implies there is a point at which the inoculum potential is so low that mycorrhizal formation is effectively precluded.

One may question whether the high incidence of nonmycorrhizal plants as invaders on disturbed land is unique to Colorado or is this a general rule in many disturbed ecosystems. The answer appears to be the latter. Allard (1965) listed the most successful noncultivated colonizers on a worldwide basis. These are: *Chenopodium album***, *Avena fatua*, *Stellaria media***, *Oxalis corniculata*, *Lactuca scariola*, *Erodium cicutarium*, *Hordeum murinum*, *Portulaca oleracea***, *Rumex crispus***, *Galinsoga* spp., *Raphanus sativus***, and *Plantago lanceolata*. Allard's conclusions are that successful colonizing species are predominately self-pollinating species. However, the relatively high incidence (42%) of potentially nonmycorrhizal species (indicated with a double asterisk) in this list deserves serious consideration. Perhaps many of these species are able and vigorous colonizers because other self-pollinating plants cannot live in the absence of mycorrhizae.

Mulligan (1965) studied the recent colonization by herbaceous plants in Canada and found that among the more common invaders were: *Thlaspi arvense***, *Linaria vulgaris*, *Chenopodium album***, *Erysimum cheiranthoides***, *Capsella bursa-pastoris***, *Raphanus raphanistrum***, *Brassica campestris***, *Spergula arvensis***, *Crepis tectorum*, *Sisymbrium loeselii***, *Centaurea repens*, *Matricaria inordora*, *Rumex* spp.**, *Carduus* spp., *Tragopogon* spp., *Ambrosia* spp., *Sonchus* spp., *Euphorbia* spp., and *Lepidium* spp.**. The high proportion (52.6%) of potentially nonmycorrhizal genera represented in this list (indicated by a double asterisk) contrasts with the observation "that most higher plants are mycotrophs" (Slankis, 1974). Pioneer species

TABLE 2. *Comparative composition, in percent mean ground cover, and mycorrhizal status of major species in natural (MES) and disturbed (DS) ecosystems*

% Mean ground cover	MES Species	Mycorrhizal status
15.77	*Artemisia tridentata*	M−
1.65	*Stipa comata*	M−
1.30	*Phlox hoodii*	M−
1.06	*Agropyron trachycaulum*	M−
0.60	*Juniperus osteosperma*	M−
0.50	*Oryzopsis hymenoides*	M−
0.36	*Agropyron smithii*	M−
0.35	*Cryptantha sericea*	M−
0.32	*Sitanion hystrix*	M−
0.26	*Koeleria cristata*	M−
0.18	*Opuntia polyacantha*	M−
0.14	*Gutierrezia sarothrae*	M−
	DS	
12.9	*Salsola kali*	M−

TABLE 3. *Vegetation succession following disturbance on semi-arid soils in the Colorado oil shale region*[a]

Ecosystem		1st year		8th year
Low elevation sagebrush reseeded with Crested wheatgrass (*Agropyron smithii*)	51%	Russian thistle (*Salsola kali*)**[b]	48%	Crested wheatgrass (*A. smithii*)
	23%	Crested wheatgrass (*Agropyron smithii*)	17%	Russian thistle (*S. kali*)**
	11%	Indian ricegrass (*Oryzopsis hymenoides*)		
Low elevation sagebrush not reseeded	41%	Russian thistle (*S. kali*)**; Lambsquarters (*Chenopodium album*)**; Miner's candle (*Cryptantha sericea*)		no data available
	34%	Indian ricegrass (*O. hymenoides*) and other perennial grasses		
	8%	Native grasses		
High elevation sagebrush reseeded with Crested wheatgrass (*A. smithii*)	50%	Russian thistle (*S. kali*)**	40%	Big sagebrush (*Artemisia tridentata*)
	22%	Indian ricegrass (*O. hymenoides*)	15%	Crested wheatgrass (*A. smithii*)
	12%	Beardless wheatgrass (*Agropyron inerme*)	9%	Beardless wheatgrass (*A. inerme*)
			17%	Knotweed (*Polygonum aviculare*)**; Stickseed (*Lappula redowskii*); Dandelion (*Taraxacum officinale*)
Bottomland sagebrush reseeded with Crested wheatgrass (*A. smithii*)	99%	Crested wheatgrass (*A. smithii*)	24%	Crested wheatgrass (*A. smithii*)
			10%	Cheatgrass (*Bromus tectorum*)
			43%	Rubber rabbitbush (*Chrysothamnus nauseosus*); Grease wood (*Sarcobatus vermiculatus*)**
			prominent	Pepperwood (*Lepidium* spp.)**; Lambsquarters (*C. album*)**; Stickseed (*L. redowskii*)
Bottomland sagebrush not reseeded	99%	Russian thistle (*S. kali*)**		no data available
	1%	Lambsquarters (*C. album*)**		

[a] Data taken from Terwilliger, Cook, and Sims, 1974.
[b] Species indicated with a double asterisk (**) are in families reported to be nonmycorrhizal (Gerdemann, 1968).

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often are exceptions to the generalization that most plants are mycorrhizal.

A tabulation of data on primary succession on certain volcanic islands further suggests a close relationship of absence of mycorrhizal fungi (sterile "soil") and traditionally nonmycorrhizal plants as primary invaders. Diamond (1977) has pointed out that Motmot Island on Long Island (near New Guinea) offers an especially interesting opportunity for studying colonization of effectively sterile habitats. The tropical location of Long Island provides a large potential group of colonists. Since the island has had only limited visits by humans, the chances for species contamination (introduction) is low. The succession of predominant higher plants on Long Island is reported by Ball and Glucksman (1975). The first-year plants all were members of the Cyperaceae. By the third year the common plants were members of the Cyperaceae, Polygonaceae, Amaranthaceae, and Urticaceae with less than ten specimens of the Compositae, Moraceae, Scrophulariaceae, and Solanaceae. By the fourth year the most abundant plants were in the Cyperaceae and Urticaceae with a few examples of the Amaranthaceae, Gramineae, Moraceae, Polygonaceae, and Ulmaceae. In summary, the early colonizers are mainly members of the Cyperaceae, Polygonaceae, Amaranthaceae, and Urticaceae. These same families are included in the list of families [compiled by Gerdemann (1968)] reported to be typically nonmycorrhizal.

In a totally different environment (Iceland) but a similar situation, viz., colonization of the volcanic island Surtsey, Iceland, a successional pattern of plants in typically nonmycorrhizal families occurs (data tabulated from Lindroth et al., 1973). On Surtsey the primary invaders are members of the Brassicaceae and Caryophyllaceae (both listed as typically nonmycorrhizal families) with a few less successful individuals of the Gramineae and Boraginaceae. By the fifth year following eruption the predominant plant was *Honckenya peploides*, a member of the Caryophyllaceae.

Mayr (1965) noted an apparent paradox, viz., "Why are colonizers normally competitively so unsuccessful in undisturbed areas? . . . Why is their success limited to such specific conditions as . . . to move into disturbed habitats and man's environment?" Mayr suggests a general answer is that "adaptive superiority is usually brought at the expense of giving up some other components of fitness, some other type of adaptive qualifications." For certain higher plants we may have a more specific answer. We suggest that data indicate that mycorrhizal plants are more competitive than nonmycorrhizal plants—mycorrhizal plants form the major species in climax and subclimax ecosystems. If, however, mycorrhizal fungi are not present (or are substantially reduced in an area) then nonmycorrhizal plants are the first to invade such sites. Often disturbed habitats result in reduced numbers of mycorrhizal propagules because the hosts themselves are reduced: there is, then, less infection. The "adaptive superiority" of the invader plants is the ability to live without mycorrhizal fungi, but the "components of fitness" they have given up is their relative inability to compete in natural ecosystems wherein most of the plants have the added fitness of mycorrhizal contributions to growth and survival.

Our working hypotheses for continued study of rehabilitation on oil shale lands may be outlined as follows: 1) Disturbance of soil leads to reduction and possibly elimination of propagules of mycorrhizal fungi (because host plants are reduced in numbers); 2) Reduced members of propagules leads to a lower potential for infection of new host plants; 3) Nonmycorrhizal species become established because normally mycorrhizal plants die in the seedling stage (for lack of mycorrhizal fungi); 4) Success of nonmycorrhizal species further reduces the propagules of mycorrhizal fungi since the fungi are obligate symbionts; 5) Total elimination of mycorrhizal fungi obviates competition by mycorrhizal higher plants; 6) Succession is slowed because of the lack of potential mycorrhizal fungi (these fungi may be slow invaders); and 7) The harsher the site the greater the potential for elimination of mycorrhizal propagules and, therefore, a longer time is required for re-establishment of mycorrhizal vegetation.

If any of these working hypotheses are correct then they have important implications for natural plant succession in various environments and especially for rehabilitation practices following energy extraction. The revegetation studies which somewhat parallel those associated with oil shale problems are those on coal wastes. Here the spoils are effectively sterile since they are from deep within the earth. Early studies (Schramm, 1966) on Pennsylvania coal spoils indicated that vegetation cover is extremely difficult to establish on some spoils. Schramm indicated that the trees established on coal wastes were mycorrhizal, and he suggested that this association was extremely important for success. Nicolson (1967) most clearly suggested that mycorrhizal fungi "may be a significant factor in soils of low fertility or in special situations, for example where soil sterilization is carried out as a routine measure or where attempts are being made to colonize bare areas such as sand or industrial waste." Nicolson referred to sterilization as done in greenhouses but the concept is equally valid for retorted oil shale and effectively sterile soil brought from deep mining as for coal and ores. Daft and Nicolson (1974) studied plants on coal wastes in Scotland and found that VA mycorrhizae occur on most successful plants on the mine spoils. The conclusion that VA mycorrhizal fungi are important in colonizing species is reported for Pennsylvania coal spoils (Daft and Hacskaylo, 1976), sand dunes (Nicolson, 1960), urban waste problems (Stevenson, 1964), and mine tailings (Harris and Jurgensen, 1977). More recently, Marx (1975) and Lindsey, Cress, and Aldon (1977) have suggested that endomycorrhizae may be essential for reclamation of coal-mining areas disturbed by energy extraction in the West.

Our survey of the native vegetation in western Colorado indicates that most species are mycorrhizal. However, colonizing species on disturbed land are often nonmycorrhizal. The nonmycorrhizal species may hinder successional stages in the ecosystem recovery because they do not provide an inoculum source for subsequent species which require mycorrhizal associations for survival. We therefore suggest that successful reclamation will depend on developing a protocol to select and/or maintain the essential mycorrhizal fungi in disturbed habits or develop methods to reinoculate these fungi in habits where they are absent. The success of rehabilitation projects to re-establish and maintain with minimum resource input conditions approximating those present before disturbance may well depend on our ability to manipulate the essential mycorrhizal fungi.

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THE ROLE OF ENDOMYCORRHIZAE IN REVEGETATION PRACTICES IN THE SEMI-ARID WEST. II. A BIOASSAY TO DETERMINE THE EFFECT OF LAND DISTURBANCE ON ENDOMYCORRHIZAL POPULATIONS[1]

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ABSTRACT

Populations of the endomycorrhizal fungus *Glomus fasciculatus* were significantly reduced following land disturbance in western Colorado soil. A bioassay was developed to measure changes in the endomycorrhizal population. In the bioassay, inoculum levels were measured by comparing the percentage infection in corn (*Zea mays*) root systems thirty days after planting in undisturbed or disturbed soils. The percentage infection was 2% in the disturbed soil compared to 77% in the adjacent undisturbed soil. *Glomus fasciculatus* was identified as the endophyte in both soils. Considering the importance and function of endomycorrhizal fungi to their plant hosts the reduction of active inoculum in the disturbed soil may be an important ecological factor in subsequent succession.

THE PRESENCE of vesicular-arbuscular endomycorrhizal fungi (VAM) has several benefits for the host plant. VA mycorrhizal plants have an increased ability to absorb phosphorus, and possibly other elements (Mosse, 1973a, b). Mycorrhizal plants may also have greater water absorption abilities (Safir, Boyer and Gerdemann, 1972). Growth increases due to VA infection have been demonstrated for many plants (Mosse, 1973a, b; Gerdemann, 1975).

VA mycorrhizal associations have been observed in a wide variety of natural and agricultural ecosystems (Read, Koucheki, and Hodgson, 1976; Williams and Aldon, 1976; Redhead, 1977; Johnson, 1977; Gerdemann, 1976; Sparling and Tinker, 1975). It has been suggested that the presence of VA mycorrhizal fungi is important in the rehabilitation of semi-arid ecosystems of the southwest United States, since these environments are often low in plant available phosphate (Williams and Aldon, 1976). Daft, Hacskaylo, and Nicolson (1975) suggested that VAM fungi might be important in the establishment of pioneer plants on coal mine wastes in Scotland and Pennsylvania. Marx and Bryan (1975) found that the introduction of ectomycorrhizal fungi greatly increased plant survival and growth in the revegetation of disturbed lands in the southeast United States. Similarly Aldon (1975) showed that endomycorrhizae increased the height, dry weight and percentage survival of *Atriplex canescens* (Fourwing Saltbush) transplanted on the New Mexico coal mine spoils. Similar results were obtained with Rabbitbrush (*Chrysothamnus nauseosus*) grown on the same material (Lindsey, Cress and Aldon, 1977).

VA mycorrhizal fungi survive in the soil in plant roots or as spores in the soil. Spore numbers and distributions in various soils were reviewed by Mosse (1973a). Spore numbers in soil have been one measurement of VAM fungi levels in the soil. However, the correlation between spore numbers in the soil and infection in plant hosts is low in some situations (perhaps because of nonviable spores) while strong in others (Hayman, 1970; Daft and Nicolson, 1972; Read et al., 1976; Redhead, 1977; Furlan and Fortin, 1977). Read et al. (1976) suggested that spores were not the principal source of inoculum, but that infected plant roots or mycelium in the soil were important sources of inocula. To overcome the difficulties intrinsic in using spore numbers as an estimate of the population, a bioassay for specific soils was developed.

The bioassay consists of determining the percentage infection in corn root systems grown for thirty days in soil from either the disturbed or undistrubed sites. Corn was selected as the bioassay plant because of its ability to serve as a host to many of the Endogonaceae (Gerdemann and Trappe, 1974). In the bioassay, only live inoculum units (spores and infected roots) are measured, whereas in spore counts viable and nonviable spores were not distinguished.

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MATERIALS AND METHODS—Fifteen-cm deep soil samples were taken from two sites in the Piceance Basin of Colorado during late November, 1976. One site is typical of the region's middle elevation sagebrush community (MES) (Ward, Slauson and Dix, 1974). The other site is a disturbed strip (DS) of land which was formerly a county road. In 1973 the road was abandoned, ripped by caterpillar tractor and left to nature. The soil samples were collected at 20-ft intervals along 200-ft transects at each site. Samples in the disturbed site (DS) were taken from the middle of the road to avoid any contamination from the edges. The samples were composited and mixed for each site, then stored in a cold chamber at 4°C.

Soils, undiluted, 1/4 and 1/40 dilutions from the MES and DS sites were assayed. The dilutions were made on a volume basis using a 1:1:1 mixture of perlite, vermiculite and sand which had been previously sterilized. Corn was planted in surface sterilized 9-cm square pots containing approximately 400 ml of these media. Controls were made by sterilizing soil from each treatment in an autoclave (twice for 1.5 hr). Each of the eight treatments contained 15 pots with one plant each to allow five plants to be harvested 30, 60 and 90 days after planting. Prior to planting all seeds were washed and sterilized for 10 min in 70% ethyl alcohol.

To assay for infection, the roots of five plants from each treatment were washed, then cut into small pieces and mixed. One hundred randomly selected 1-cm root sections were removed from each bulk sample and stained according to the method of Phillips and Hayman (1970). The stained root sections were mounted in lactophenol and examined under a compound microscope. The percentage infection was calculated as the number of segments with any infection out of the sample of 100 (Daft and Nicolson, 1966). Only those segments containing mycorrhizal hyphae and either vesicles or arbuscules were counted as infected.

Soil analysis of the MES and DS soil was done in the Colorado State University Soil Testing Laboratory. Spores of VA mycorrhizal fungi were counted in the soil from each site. Eight counts at each site were made by use of a modified wet sieving method (Gerdemann, 1955). Endomycorrhizal fungi were identified according to Gerdemann and Trappe (1974).

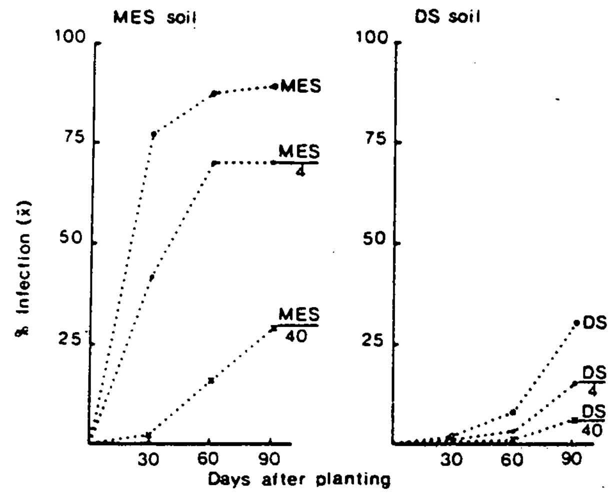

Fig. 1. A comparison of mean percent infection in corn roots grown for 30, 60 and 90 days in MES and DS undiluted and diluted (1/4 and 1/40) soils.

RESULTS—The mean infection percentages of the bioassay plants 30, 60 and 90 days after planting are given in Table 1 and shown in Fig. 1. At the 30 day sampling time the MES plants were 77% infected, whereas the DS plants were only 1% infected, indicating a greatly reduced amount of inoculum in the DS soil. The effect of dilution was to reduce the amount of infection in the MES plants accordingly. Diluting the DS soil also reduced mean infection, but because of the low inoculum density in this soil, the effects of dilution do not become apparent until 60–90 days.

The most common endophyte present in wet-sieved soil and the bioassay plants was *Glomus fasciculatus*. However, spores identified as *G. microcarpus* were occasionally observed.

The growth of *Glomus fasciculatus* within the corn roots followed a pattern typical of mycorrhizae in summer crops (Saif, 1977). At the thirty day sampling period the corn roots had penetrated all parts of the pot, thus encountering most potential inoculum units. At this time abundant arbuscules could be seen in the cortical cells, but only a few vesicles. Sixty days after planting numerous vesicles could be seen with the arbuscules. Spores were observed attached to external mycelium in the roots of plants harvested 90 days after planting. No sporocarps were observed.

The results of total spore counts are presented in Table 2. Surprisingly, the DS soil contains significantly greater numbers of spores of VA mycorrhizal fungi, but many of these spores observed under a compound microscope were found to be cracked or dead. An average of 6.7 spores per g in the MES soil was found compared to an average of 10.4 spores in the DS soil. These counts are significantly different at the 0.01 level.

The results of the soil analyses are given in Table 3.

DISCUSSION—At this time, no accurate method for the quantification of VA mycorrhizal fungi

TABLE 1. *Comparison of percentage infection of bioassay plants grown in undisturbed (MES) vs. disturbed (DS) soil*

Treatment	Sampling time[a]								
	30 days			60 days			90 days		
MES soil	66[b]	88[c]	77[d]	85	89	87	80	97	89
1/4 MES soil	37	46	42	57	82	70	45	94	70
1/40 MES soil	0	3	2	17	15	16	37	20	29
DS soil	2	0	1	0	15	8	17	42	30
1/4 DS soil	1	0	1	7	0	4	17	15	16
1/40 DS soil	1	0	1	2	0	1	5	7	6
Control	0	0	0	0	0	0	0	0	0

[a] Days after planting.
[b] Replication 1.
[c] Replication 2.
[d] Mean infection.

has been developed. Spore counts are the most common method for quantifying VA mycorrhizal inoculum in the soil, but this is inconclusive due to the effects of plant nutrients, season, host plant, and possibly other factors on the production of spores. Spores are not the only structures that can serve as inoculum units (Powell, 1976); endomycorrhizal roots also can act as inocula. Redhead (1977) was unable to extract spores from a Nigerian rain forest soil and a New Zealand bush soil where infected plants were found. Similarly our data show an inconsistency between spore numbers and mycorrhizal development in a host plant on the disturbed land (DS).

The bioassay measures the realtive amounts of inoculum in the soils by observing the amount of development of the mycorrhizal fungi in the host plants. Our system utilized corn which is known to be a host to many VA mycorrhizal fungi. The bioassay is probably most accurate 30 days after planting since infection is mainly from propagules and not from growth of the fungus within the roots. However, in other situations these conditions may need to be modified to accurately bioassay the relative amounts of mycorrhizal inoculum in soils.

The differences in the percentage infection thirty days after planting in the DS and MES bioassay plants probably results from a reduction in the inoculum potential in the DS. This is expected since the DS exhibits a low incidence of mycorrhizal plants (Reeves et al., 1978). High levels of available nitrogen and phosphorus can reduce the amount of infection by the Endogonaceae, but the levels of these nutrients are low in both soils (Table 3). Zinc has been demonstrated to inhibit the growth of *Endogone* sp. in pinto beans, and to inhibit the germination of spores in petri plates (McIlveen, Spotts, and Davis, 1975; Hepper and Smith, 1976). It seems unlikely that the higher Zn concentration in the DS soil alone could account for these results. A Sodium Adsorption Ratio (SAR) value of 11 represents the lowest end of a moderate sodium hazard range to higher plants. Information on the effects of sodium on soil fungi or mycorrhizae is sparse (Richards, 1954). The effect of sodium at the DS would be to reduce water infiltration due to soil dispersion, but effects of this kind would be minimized by the bioassay methods.

The reduction of populations of mycorrhizal fungi may be significant in the re-establishment of stable ecosystems on disturbed lands. The low populations of mycorrhizal fungi found in the DS soil may not allow the plant hosts to become sufficiently infected to receive the growth stimulation that often accompanies normal infection levels. Some support for this idea is expressed by Daft and Nicolson (1969) in their work on the influence of mycorrhizal inoculum concentrations and the growth of tomatoes. Plants receiving the largest amounts of inoculum exhibited

TABLE 2. *Comparison of endomycorrhizal spore counts from two Colorado soils*

Soil	Count number								Mean
	1	2	3	4	5	6	7	8	
MES soil	20[a]	26	26	33	40	36	39	48	6.7[b]
DS soil	53	48	48	43	66	58	40	59	10.4

[a] Spores per 5 g soil sample.
[b] Mean expressed per 1 g soil sample.

TABLE 3. *Chemical characteristics of two Colorado soils*

Soil	pH	Cond.[a] mmhos/cm	O.M.[b] %	P ppm	K ppm	NO_3 ppm	Zn ppm	Fe ppm	SAR[c]
MES soil	8.0	0.5	1.1	3	95	1	0.5	2.4	0.3
DS soil	8.2	1.6	0.7	5	99	13	3.2	3.7	11.2

[a] Electrical conductivity, a measure of total soluble salts.
[b] Organic matter.
[c] Sodium Adsorption Ratio, a measure of exchangeable sodium ions.

growth increases more quickly after planting than those plants receiving lesser amounts of inoculum.

We estimate that the viable populations in the DS are approximately 1/40 that of the MES. This is indicated by the roughly equal infection percentages in the 1/40 dilution of MES soil and the DS soil. The bioassay is a more accurate assessment of the populations at the two sites than spore counts which show greater numbers of spores in the DS soil. The bioassay technique offers an alternative method for comparing populations in soils, wherein only the active VA mycorrhizal fungal propagules, under variable soil conditions, are measured.

Additional research on the suitability of corn as a bioassay host and the environmental effects (light, temperature, etc.) on percent infection is needed. Quite likely several other hosts with wider symbiotic relationships may provide a more rapid or more accurate method for other soils or other conditions.

Certainly there is a strong correlation between the incidence of active mycorrhizal propagules and the incidence of mycorrhizal plants in these soils (Reeves et al., 1978). This correlation has far reaching implications in studying succession on severely disturbed habits and in planning reclamation efforts on disturbed lands. Perhaps both quantitative (number of viable propagules) and qualitative (presence or absence of particular species) aspects of mycorrhizal infection potential must be considered in rehabilitation programs for disturbed lands. Current research is underway to determine the relative impact of various soil disturbances on mycorrhizal potential.

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APPENDIX A

P Tables

Table 1. P values from analysis of variance on biomass of seeded, invading, and total (seeded and invading) species from the Revegetation Technique Plot.

Treatment	Biomass[1]		
	Seeded	Invading	Total
Irrigation	.1490	.0986	.1406
Seed Mix	.1603	.1236	.1811
Irrigation x Seed Mix	.1012	.1699	.2303
Seeding Technique	.2142	.4411	.1518
Irrigation x Seeding Technique	.2279	.5313	.1723
Seed Mix x Seeding Technique	.4983	.8417	.4672
Irrigation x Seed Mix x Seeding Technique	.4812	.7685	.4216
Fertilization	.0016	.0033	.0003
Irrigation x Fertilization	.8413	.0166	.5144
Seed Mix x Fertilization	.6073	.0137	.5211
Irrigation x Seed Mix x Fertilization	.1263	.0286	.2653
Seeding Technique x Fertilization	.9823	.3985	.8986
Irrigation x Seeding Technique x Fertilization	.0471	.4241	.0567
Seed Mix x Seeding Technqiue x Fertilization	.4657	.9731	.4638
Irrigation x Seed Mix x Seeding Technique x Fertilization	.7760	.7643	.6970

[1]All P values ≤ .05 were considered significant.

Table 2. P values from analysis of variance on the Revegetation Technique Plot on Deeply Disturbed Soils. Each life form was analyzed separately. This data represents biomass from seeded species.

Treatment	Biomass[1]		
	Grasses	Forbs	Shrubs
Irrigation	.0559	.0607	.8858
Seed Mix	.0004	.0081	.0278
Irrigation x Seed Mix	.0067	.0147	.4828
Seeding Technique	.0002	.3368	.0267
Irrigation x Seeding Technique	.1265	.6656	.0605
Seed Mix x Seeding Technique	.0353	.3009	.2420
Irrigation x Seed Mix x Technique	.6751	.3460	.3603
Fertilization	.0000	.6230	.1838
Irrigation x Fertilization	.0000	.9972	.1772
Seed Mix x Fertilization	.6250	.9726	.5757
Technique x Fertilization	.0582	.5357	.3560
Irrigation x Seed Mix x Fertilization	.5645	.6831	.1118
Seed Mix x Technique x Fertilization	.8830	.5187	.7528
Irrigation x Technqiue x Fertilization	.0851	.7053	.3397
Irrigation x Seed Mix x Technqiue x Fertilization	.9520	.4814	.7604

[1]All P values ≤ .05 were considered significant.

Table 3. P values from analysis of variance on the Revegetation Technique Plot on Deeply Disturbed Soils. Each life form was analyzed separately. This data represents biomass of invaders.

Treatment	Biomass[1]		
	Grasses	Forbs	Shrubs
Irrigation	.1418	.2147	.2570
Seed Mix	.1494	.0928	.2726
Irrigation x Seed Mix	.3897	.2138	.2655
Seeding Technique	.8558	.0816	.5520
Irrigation x Technique	.6521	.1361	.4618
Seed Mix x Technique	.2764	.1302	.9253
Irrigation x Seed Mix x Technique	.6587	.1030	.9057
Fertilization	.0373	.0487	.6637
Irrigation x Fertilization	.2589	.2124	.6180
Seed Mix x Fertilization	.2182	.0394	.8974
Technique x Fertilization	.1090	.4037	.3883
Irrigation x Seed Mix x Fertilization	.2127	.1577	.8570
Seed Mix x Technique x Fertilization	.7936	.6596	.6154
Irrigation x Technique x Fertilization	.0433	.5093	.4238
Irrigation x Seed Mix x Technique x Fertilization	.2809	.6290	.5922

[1]All P values ≤ .05 were considered significant.

Table 4. P values from analysis of variance on the Successional Study on Surface Disturbed Soil. Each life form was analyzed separately. This data represents density of seeded species.

Treatment	Density[1]		
	Grasses	Forbs	Shrubs
Mulch	.0225	.1336	.3036
Seed Mix	.0000	.0525	.1864
Mulch x Seed Mix	.3441	.8102	.2012
Fertilization	.7446	.1601	.5682
Seed Mix x Fertilization	.7335	.1252	.1058
Mulch x Fertilization	.8095	.9235	.5356
Seed Mix x Mulch x Fertilization	.0545	.3915	.0246

[1]All P values $\leq .05$ were considered significant.

Table 5. P values from anaysis of variance on the Successional Study on Surface Disturbed Soil. Each life form was analyzed separately. This data represents cover of seeded species.

Treatment	Cover[1]		
	Grasses	Forbs	Shrubs
Mulch	.5486	.9606	.4289
Seed Mix	.4908	.0220	.0102
Mulch x Seed Mix	.9618	.9885	.7265
Fertilization	.0002	.0588	.4229
Seed Mix x Fertilization	.7397	.8974	.4084
Seed Mix x Mulch x Fertilization	.5143	.0568	.4343

[1]All P values $\leq$ .05 were considered significant.

Table 6. P values from analysis of variance on the Successional Study on Surface Disturbed Soil. Each life form was analyzed separately. This data represents biomass of seeded species.

Treatment	Biomass[1]		
	Grasses	Forbs	Shrubs
Mulch	.3763	.0276	.2717
Seed Mix	.0010	.0110	.0183
Mulch x Seed Mix	.4925	.8632	.2818
Fertilization	.0025	.7611	.2763
Mulch x Fertilization	.7153	.2189	.6898
Seed Mix x Fertilization	.3781	.8566	.3149
Mulch x Seed Mix x Fertilization	.2296	.2581	.6249

[1]All P values ≤ .05 were considered significant.

Table 7. P values from analysis of variance on the Successional Study on Surface Disturbed Soil. Each life form was analyzed separately. This data represents density of invading species.

Treatment	Density[1]		
	Grasses	Forbs	Shrubs
Mulch	.6005	.3021	.5799
Seed Mix	.7545	.1859	.7232
Mulch x Seed Mix	.8631	.7939	.3754
Fertilization	.4660	.2216	.3418
Mulch x Fertilization	.9389	.4688	1.0000
Seed Mix x Fertilization	.2898	.6766	.2180
Mulch x Seed Mix x Fertilization	.7335	.2656	.3006

[1]All P values ≤ .05 were considered significant.

Table 8. P values from analysis of variance on the Successional Study on Surface Disturbed Soil. Each life form was analyzed separately. This data represents cover of invading species.

Treatment	Cover[1]		
	Grasses	Forbs	Shrubs
Mulch	.3918	.2742	.4577
Seed Mix	.0713	.0126	.2201
Mulch x Seed Mix	.0481	.5303	.1582
Fertilization	.4353	.6066	.2504
Mulch x Fertilization	.4274	.6761	.3868
Seed Mix x Fertilization	.8377	.7092	.7794
Mulch x Seed Mix x Fertilization	.5653	.1271	.5785

[1]All P values ≤ .05 were considered significant.

Table 9. P values from analysis of variance on the Retorted Shale Study. (Each life form was analyzed separately. This data represents density from seeded species only.)

Treatment	Density[1]		
	Grasses	Forbs	Shrubs
Panel	.0000	.0000	.0010
Seed Mixture	.4415	.0000	.0000
Panel x Seed Mixture	.1665	.0006	.0258
Fertilization	.9539	.2070	.1349
Panel x Fertilization	.2891	.2014	.8416
Seed Mixture x Fertilization	.4338	.4766	.4014
Panel x Seed Mixture x Fertilizer	.9887	.3410	.9124

[1]All P values $\leq$ .05 were considered significant.

Table 10. P values from analysis of variance for Retorted Shale Study. (This analysis represents the treatment response of total biomass, total cover, and total density where total is the summation of invading and seeded species.)

Treatment	Response[1]		
	Biomass	Cover	Density
Panel	.0037	.1825	.0000
Seed Mixture	.2726	.1859	.0325
Panel x Seed Mixture	.6165	.3121	.5759
Fertilization	.4522	.2898	.6555
Panel x Fertilization	.6591	.2768	.2266
Seed Mixture x Fertilization	.0779	.3987	.5819
Panel x Seed Mixture x Fertilization	.8307	.5673	.9782

[1]All P values ≤ .05 were considered significant.

Table 11. P values from analysis of variance for Retorted Shale Study. (This analysis represents total grasses, forbs, and shrubs for seeded species only.)

Treatment	Response[1]		
	Biomass	Cover	Density
Panel	.0000	.1844	.0000
Seed Mixture	.0238	.1569	.0376
Panel x Seed Mixture	.1209	.2754	.5937
Fertilization	.6574	.2931	.6452
Panel x Fertilization	.7546	.2487	.2312
Seed Mixture x Fertilization	.4827	.4426	.5722
Panel x Seed Mixture x Fertilization	.9106	.5728	.9790

[1]All P values $\leq$ .05 were considered significant.

Table 12. P values from analysis of variance on the Retorted Shale Study. (Each life form was analyzed separately. This data represents cover of seeded species only.)

Treatment	Cover[1]		
	Grasses	Forbs	Shrubs
Panel	.0005	.3708	.0676
Seed Mixture	.5409	.2855	.0045
Panel x Seed Mixture	.8781	.3766	.2066
Fertilization	.2517	.3732	.2965
Panel x Fertilization	.4652	.3721	.7061
Seed Mixture x Fertilization	.5041	.3673	.1863
Panel x Seed Mixture x Fertilization	.7048	.3831	.9921

[1]All P values ≤ .05 were considered significant.

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APPENDIX B

Soil and Meteorological Data

Table 1. Laboratory nitrogen analysis of soil taken at various depths from the panels of the Retorted Shale Successional Study, 1978.[1]

Location		Sample Depth	Percent Total N	ppm NH_4-N	ppm NO_3-N
Panel	Rep				
2	I	0-15 cm	0.071	10	5
2	I	15-30 cm	0.058	16	6
2	II	0-15 cm	0.070	6	5
2	II	15-30 cm	0.051	11	4
2	III	0-15 cm	0.068	5	2
2	III	15-30 cm	0.060	14	5
3	I	0-15 cm	0.076	4	5
3	I	15-30 cm	0.066	11	3
3	II	0-15 cm	0.075	10	4
3	II	15-30 cm	0.073	12	5
3	III	0-15 cm	0.084	6	5
3	III	15-30 cm	0.073	14	4
4	I	0-15 cm	0.084	7	5
4	I	15-30 cm	0.072	8	3
4	II	0-15 cm	0.081	10	2
4	II	15-30 cm	0.072	11	4
4	III	0-15 cm	0.084	2	5
4	III	15-30 cm	0.082	13	5
5	I	0-15 cm	0.080	4	6
5	I	15-30 cm	0.063	20	4
5	II	0-15 cm	0.062	4	6
5	II	15-30 cm	0.065	11	5
5	III	0-15 cm	0.066	3	6
5	III	15-30 cm	0.068	12	4
6	I	0-15 cm	0.084	3	5
6	I	15-30 cm	0.082	17	5
6	II	0-15 cm	0.080	4	6
6	II	15-30 cm	0.084	14	5
6	III	0-15 cm	0.081	2	4
6	III	15-30 cm	0.079	13	4

[1]Soil samples collected and analyzed in July 1978.

Table 2. Laboratory analysis of Paraho retorted shale and soil taken at various depths in the panels of the Retorted Shale Successional Study.[1] Soil samples collected and analyzed in November 1978.

Location	Sample Type	pH	Conductivity	Ca	Mg	Na	K	SAR	Meq/100 g CEC
Panel 1	Surface Shale	9.2	6.2	5.6	43.2	27.4	4.7	5.6	4.7
Panel 1	Surface Shale	9.2	5.0	5.1	31.7	22.0	3.9	5.1	5.1
Panel 1	Surface Shale	9.2	5.3	5.8	34.7	20.0	3.6	4.4	4.3
Panel 1	Subsurface Shale	9.2	8.2	21.5	31.2	49.1	5.1	9.6	5.2
Panel 1	Subsurface Shale	9.3	7.2	21.6	23.7	38.9	4.7	8.2	5.0
Panel 1	Subsurface Shale	9.3	6.7	22.8	28.9	35.3	4.8	6.9	4.3
Panel 2	Soil Above Interface	8.5	1.8	2.4	2.5	15.0	0.1	9.5	16.3
Panel 2	Soil Above Interface	8.9	2.4	2.6	3.9	18.6	<0.1	10.3	15.1
Panel 2	Soil Above Interface	8.5	1.4	2.4	1.6	12.0	<0.1	8.4	19.1
Panel 2	Soil Below Interface	9.5	5.1	24.4	13.2	22.0	1.7	5.1	7.4

Table 1.--Continued

Location	Sample Type	pH	Conductivity	Ca	Mg	Na	K	SAR	Meq/100 g CEC
Panel 2	Shale Below Interface	9.3	5.0	23.8	21.4	18.5	1.7	3.9	6.4
Panel 2	Shale Below Interface	9.4	6.3	23.0	17.6	34.8	2.2	7.7	7.2
Panel 4	Subsoil	8.7	2.4	3.0	3.8	19.8	0.1	10.7	15.2
Panel 4	Subsoil	8.5	4.3	11.7	10.7	23.3	0.2	7.0	18.9
Panel 4	Subsoil	8.5	1.3	3.3	2.1	8.6	0.1	5.2	16.0
Panel 5	Shale Below Interface	9.5	7.4	22.3	16.0	52.2	2.8	11.9	6.3
Panel 5	Shale Below Interface	9.5	7.2	22.3	20.1	43.4	3.0	9.4	6.1
Panel 5	Shale Below Interface	9.1	5.6	23.4	28.1	21.8	1.0	4.3	9.5

[1]Conductivity, pH, Ca, Mg, Na, K, and SAR were determined from a saturation extract for all soil material and from a 1:1 extract for all shale material.

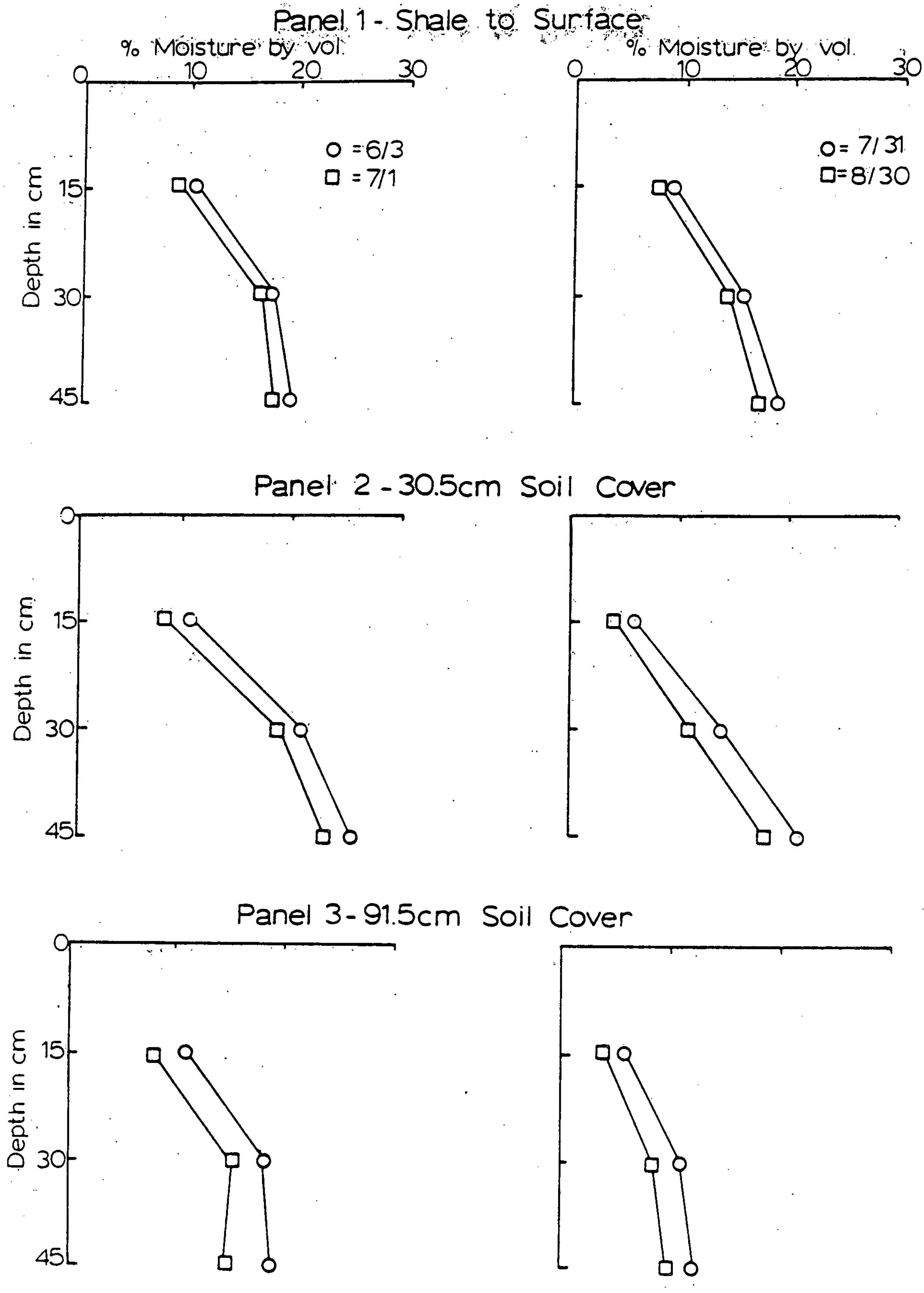

Figure 1. Subsurface soil moisture by volume for the Successional Study on Retorted Shale and Soil Over Retorted Shale. (Readings were taken on 6/3, 7/1, 7/31, and 8/30/78.)

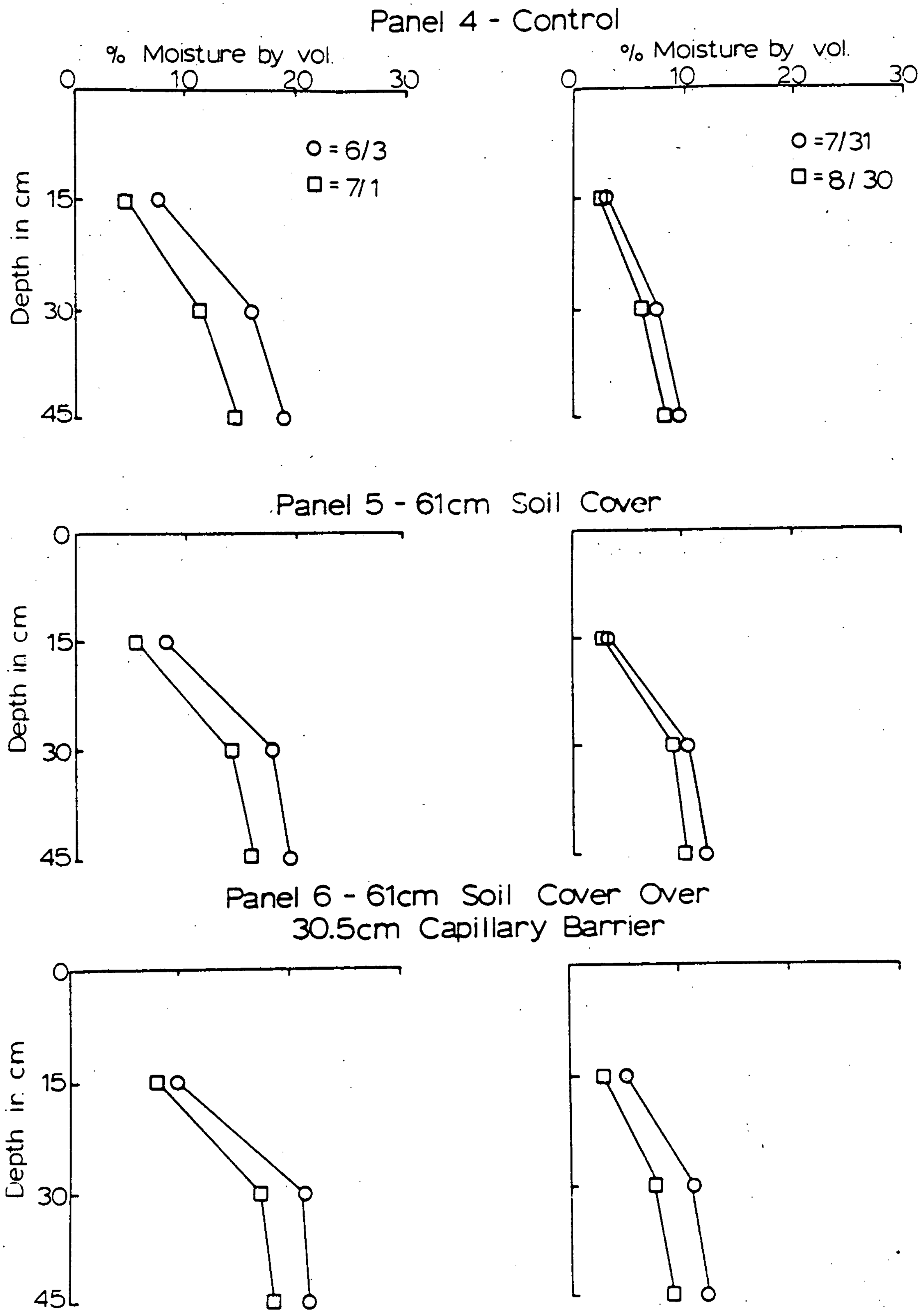

Figure 2. Subsurface soil moisture by volume for the Successional Study on Retorted Shale and Soil Over Retorted Shale. (Readings were taken on 6/3, 7/1, 7/31, and 8/30/78.)

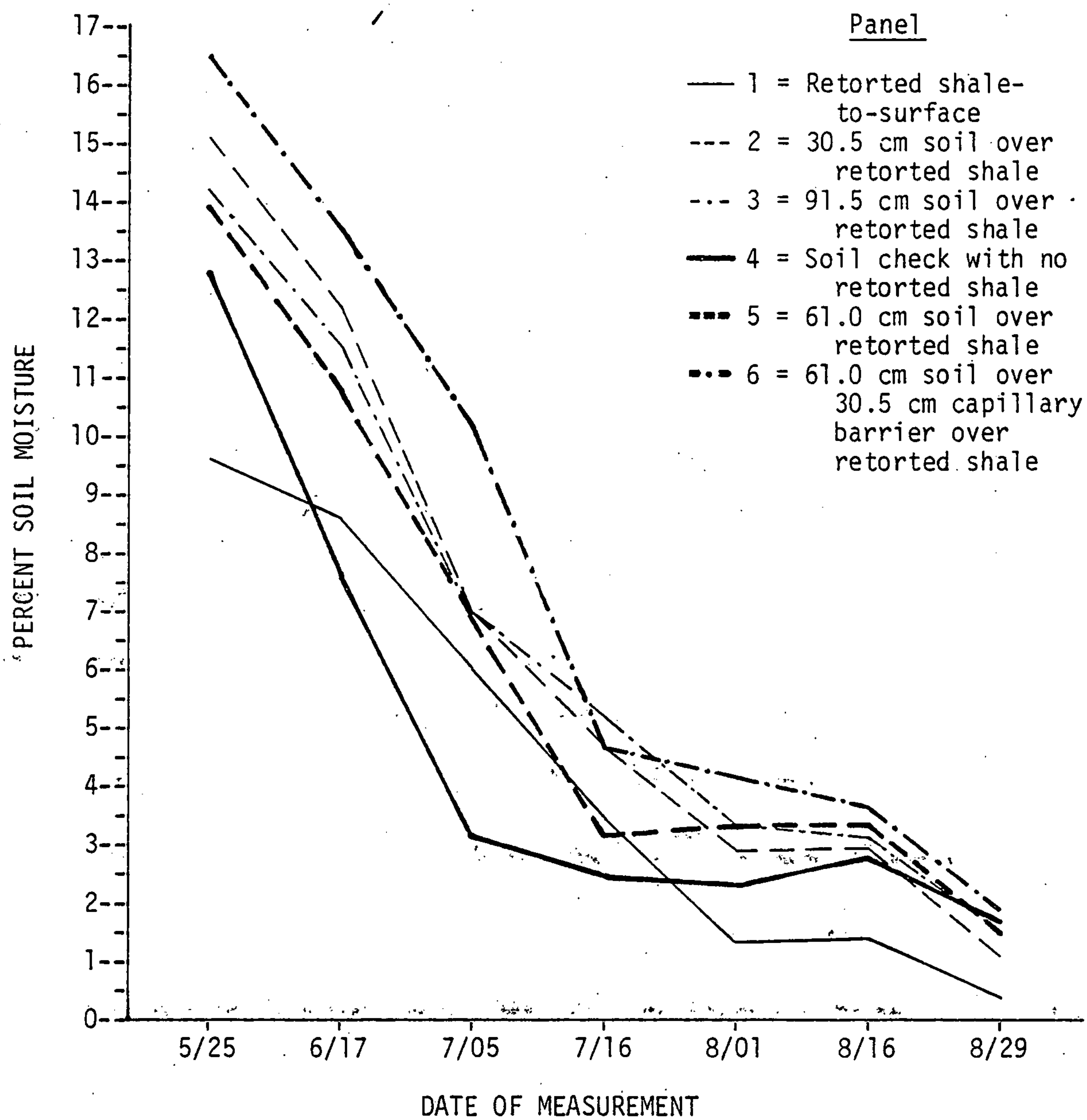

Figure 3. Percentage surface soil moisture for the Successional Study on Retorted Shale and Soil Over Retorted Shale, 1978.

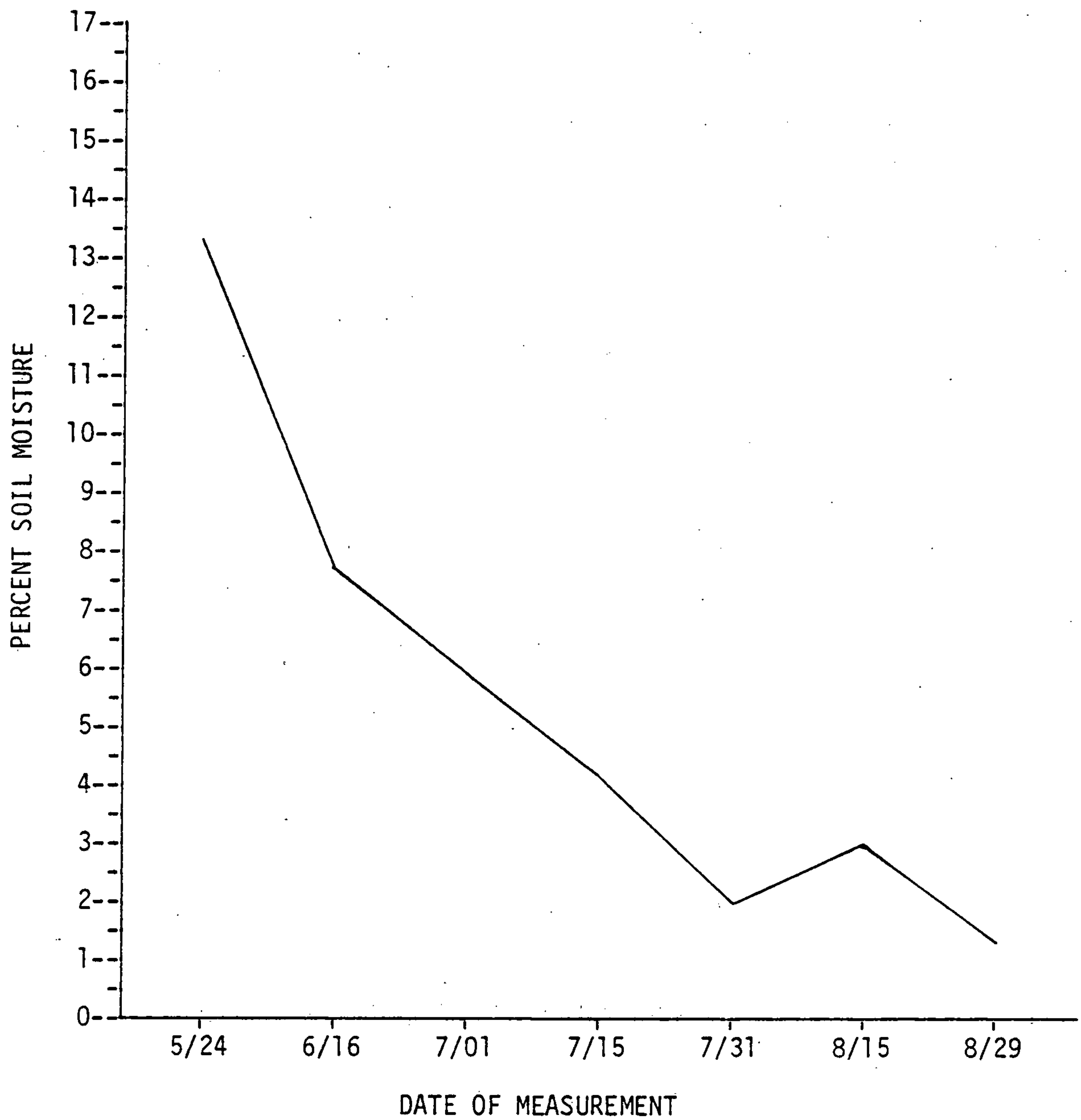

Figure 4. Percentage surface soil moisture for the Successional Study on Shallowly Disturbed Soils, 1978.

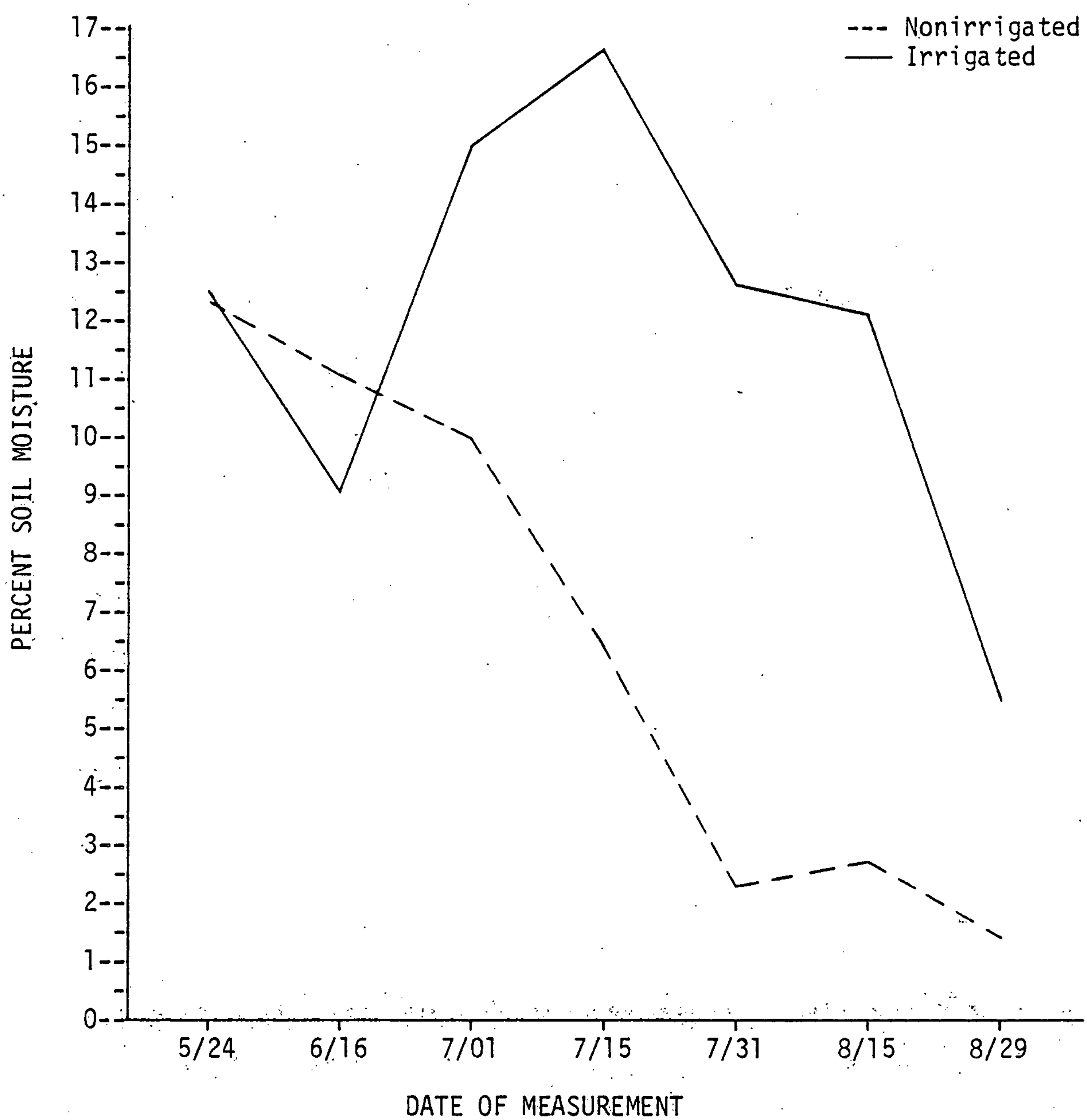

Figure 5. Percentage surface soil moisture for the Revegetation Technique Plot on Intensively Disturbed Soils, 1978.

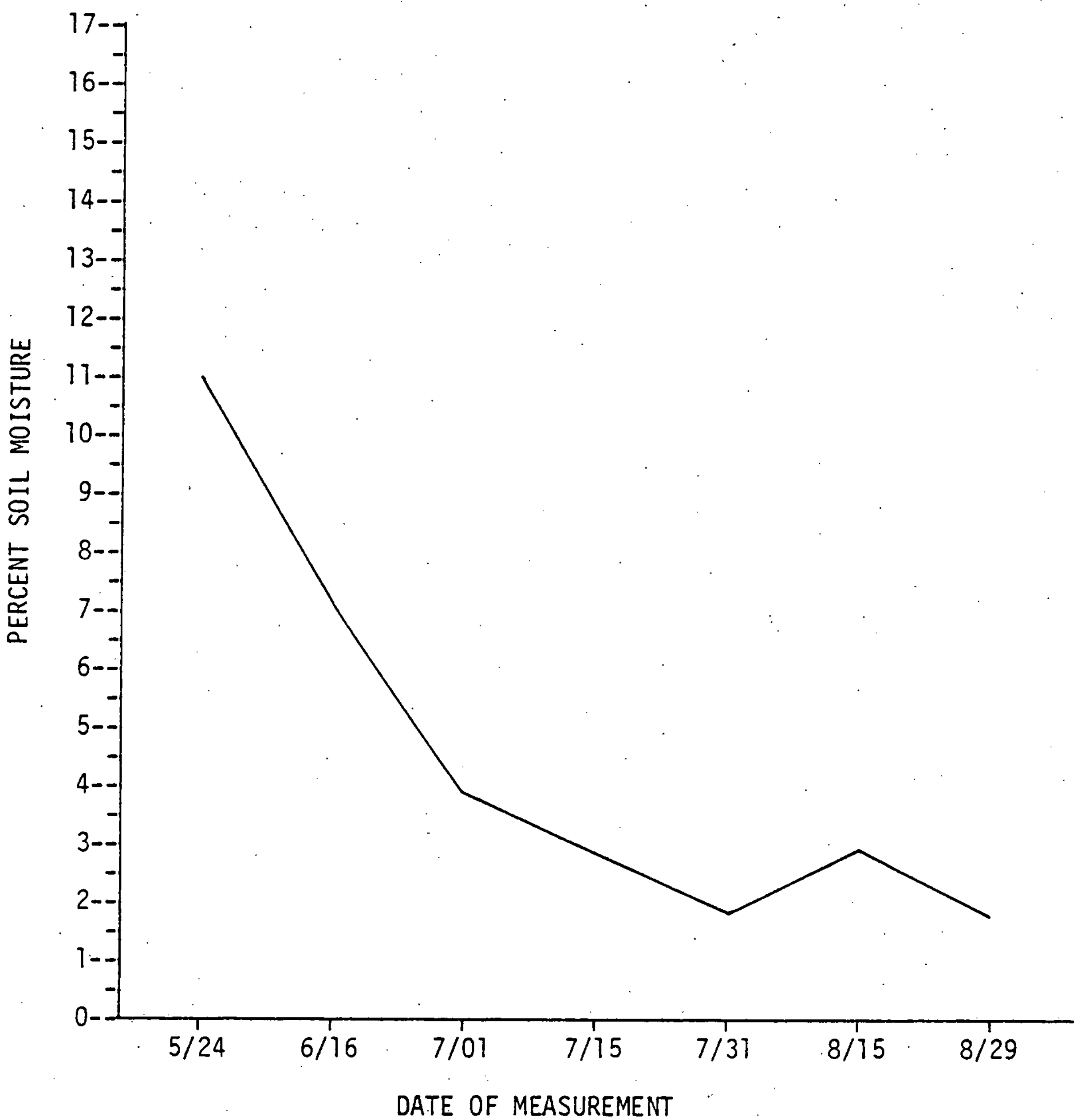

Figure 6. Percentage surface soil moisture for the Undisturbed Native Sagebrush Community, 1978.

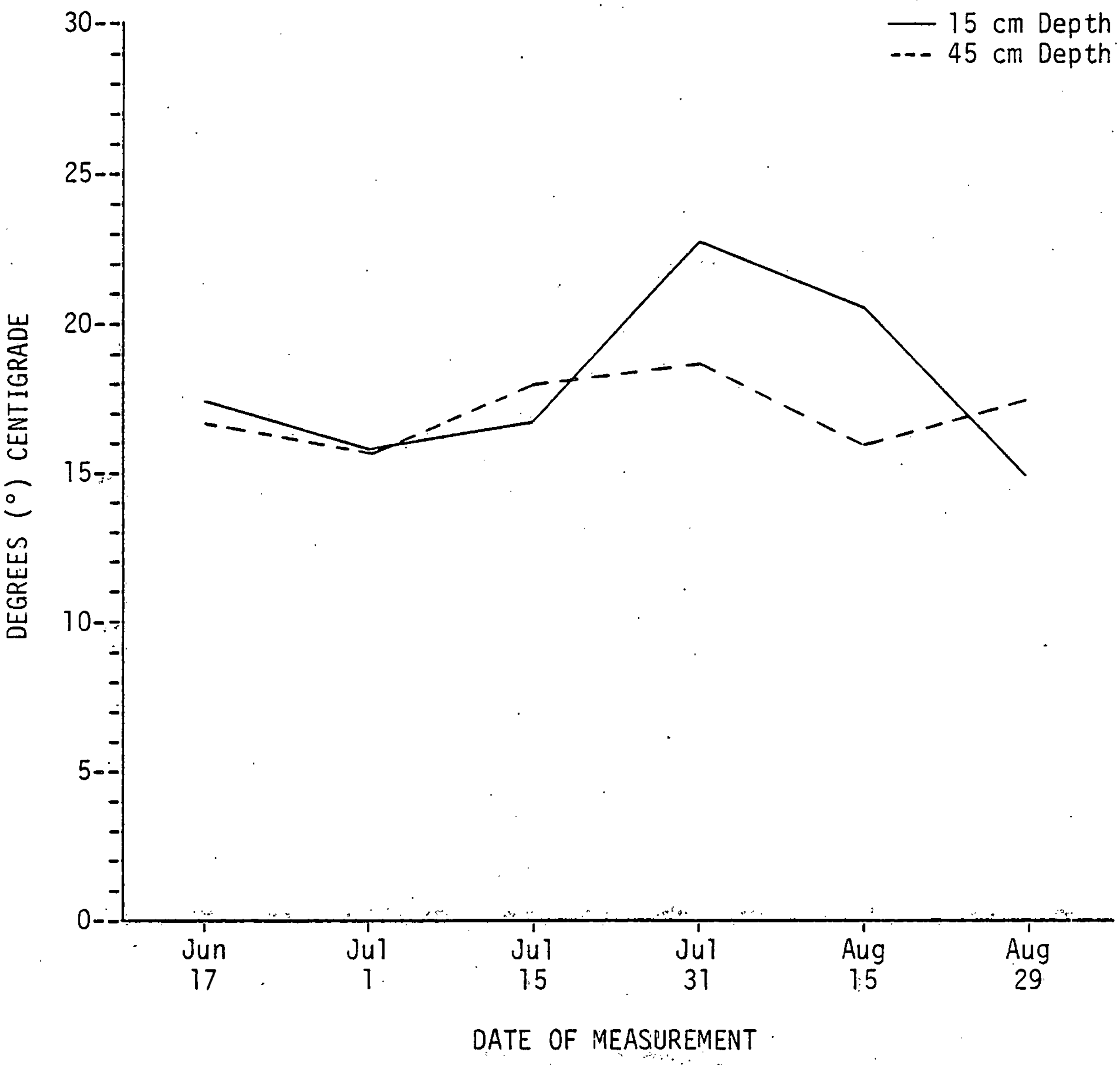

Figure 7. Mean soil temperature for the irrigated section of the Revegetation Technique Plot on Intensively Disturbed Soils, 1978.

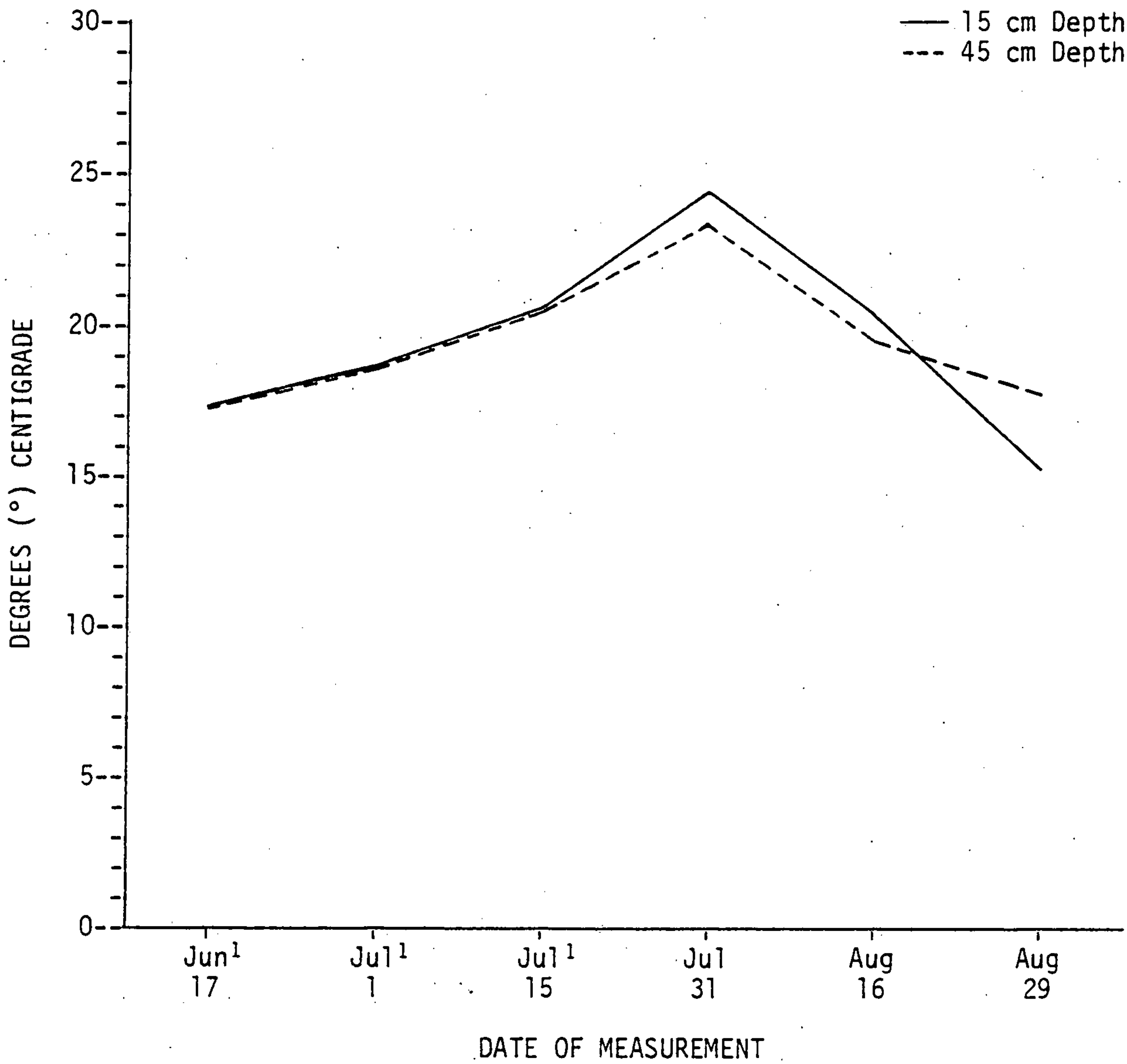

Figure 8. Mean soil temperature for the nonirrigated section of the Revegetation Technique Plot on Intensively Disturbed Soils, 1978.

[1]The measurements on these dates are the same for both 15 and 45 cm depths.

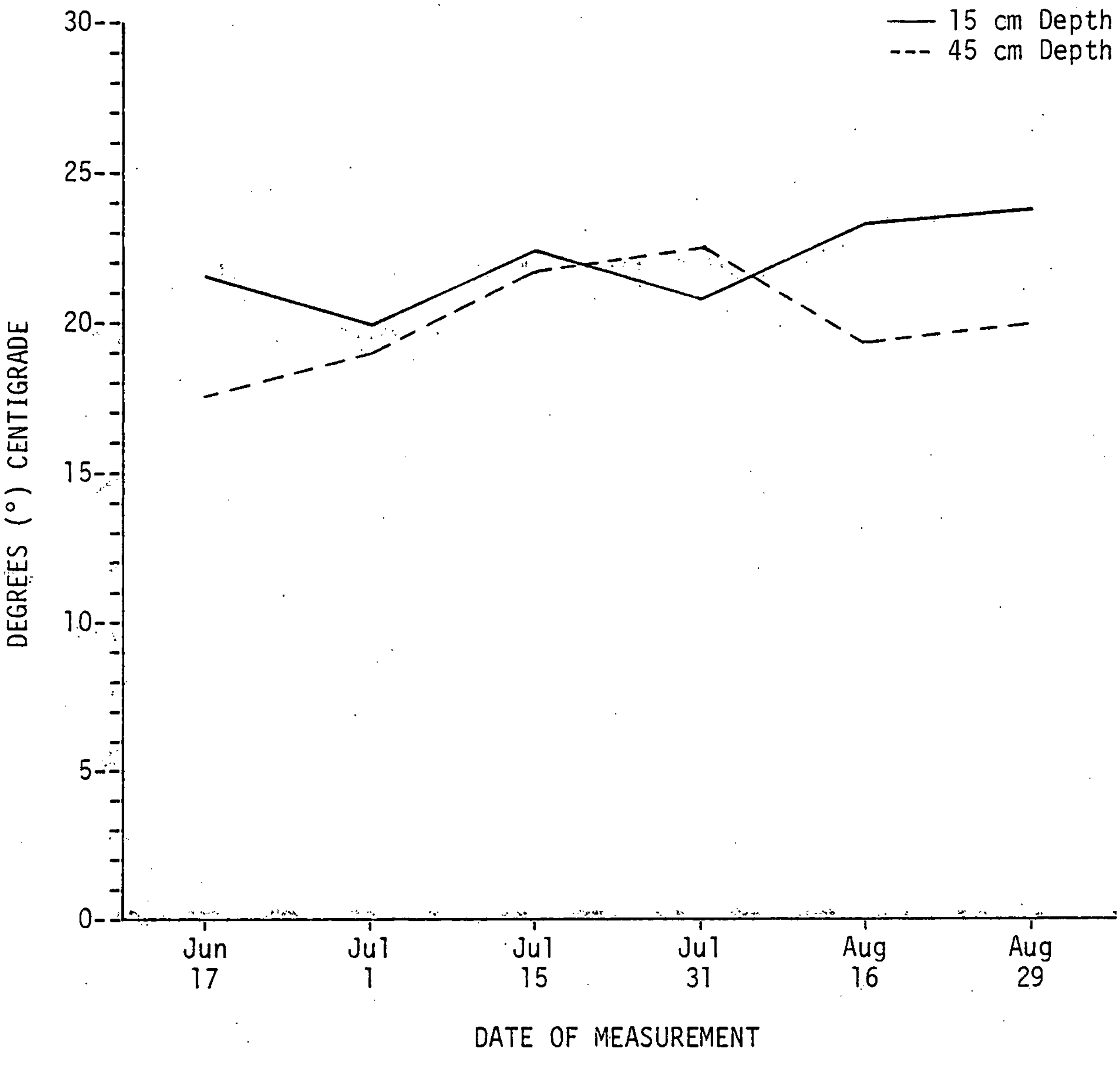

Figure 9. Mean soil temperature for the Undisturbed Sagebrush Community, 1978.

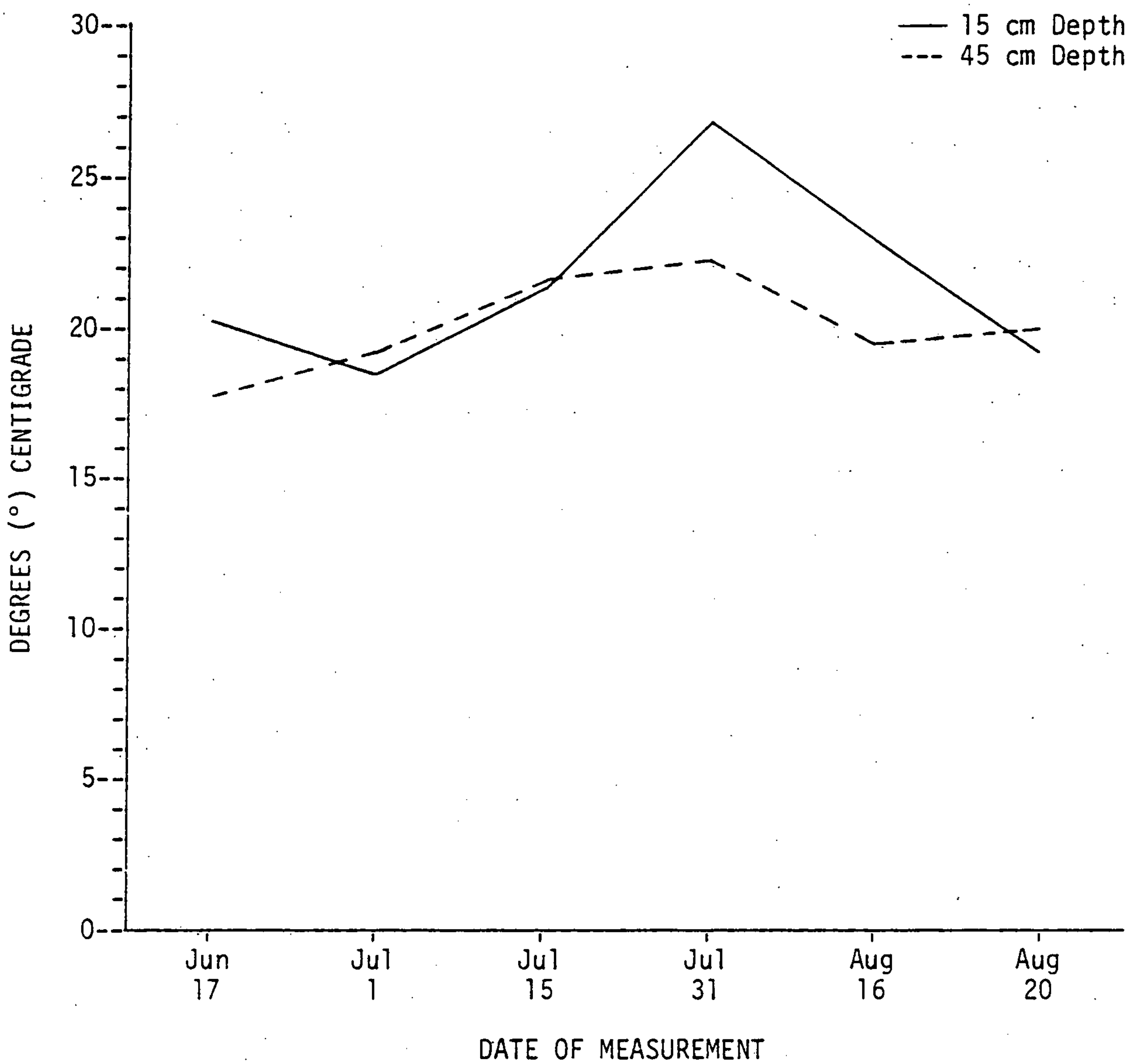

Figure 10. Mean soil temperature for the Successional Plot on Shallowly Disturbed Soils, 1978.

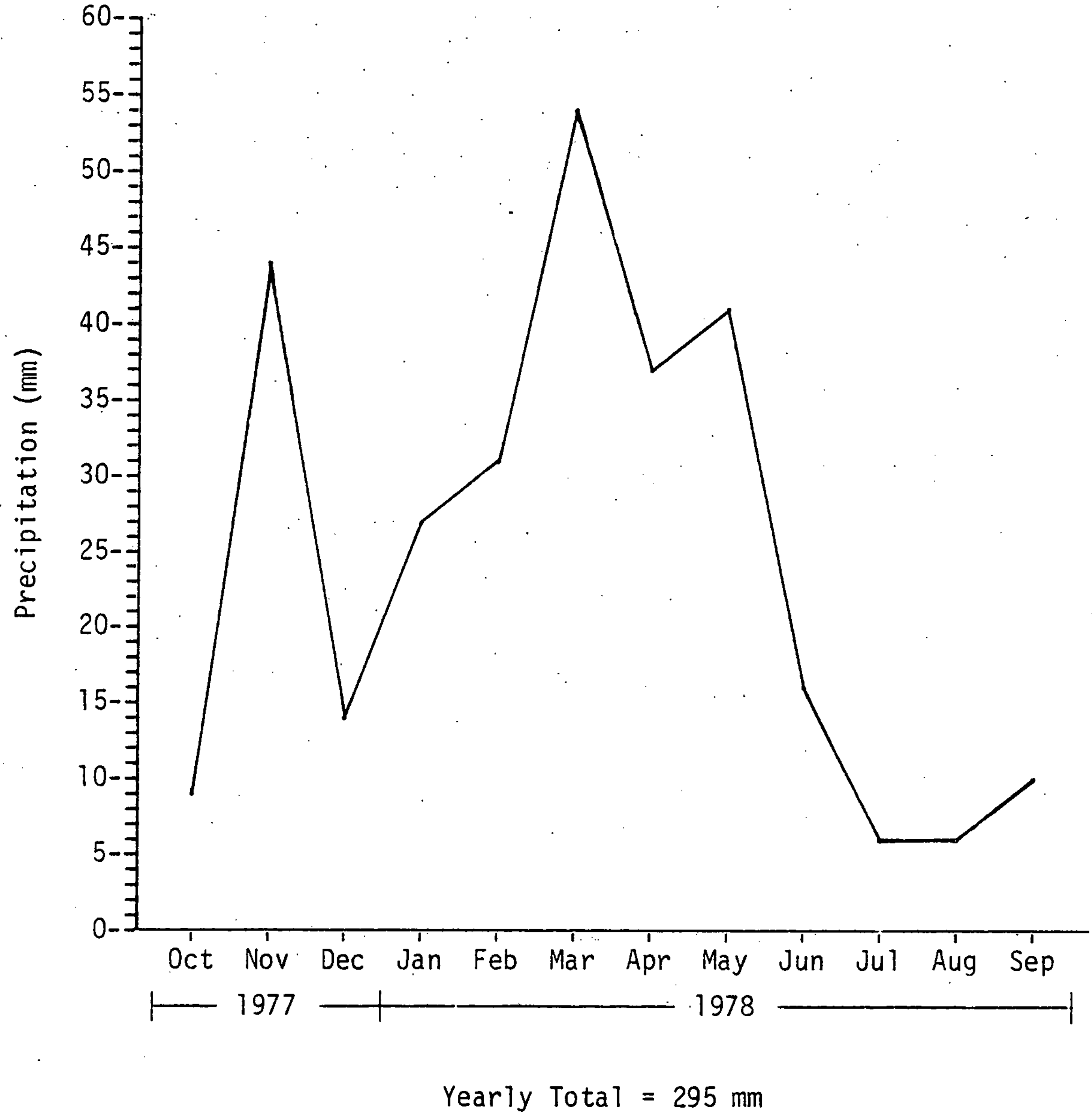

Figure 11. Precipitation data recorded (mm) at the Intensive Study Site.